This is to certify that the

thesis entitled

RIPENING BEHAVIOR OF WILD TOMATO SPECIES

presented by

REBECCA GRUMET

has been accepted towards fulfillment
of the requirements for

M.S. degree in Horticulture

Jon F. Fobes
Major professor

Date April 28, 1980

RIPENING BEHAVIOR OF WILD TOMATO SPECIES

By

Rebecca Grumet

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

1980

ABSTRACT

RIPENING BEHAVIOR IN WILD TOMATO SPECIES

By

Rebecca Grumet

Nine wild tomato species were surveyed for variability in ripening habit. Fruits harvested at different ages were examined for ethylene production, respiratory behavior, and external ripening changes. Ethylene production and CO_2 evolution were monitored using an ethylene-free flow-through system and gas chromatography.

The three color-fruited species all exhibited typical climacteric behavior in correspondence with visible ripening changes. Variability was observed for timing and magnitude of the climacteric response. Two of the green-fruited species did not ripen prior to abscission. They differed, however, for timing of endogenous ethylene production relative to abscission. The other green-fruited species ripened on the vine. They varied with respect to ethylene production, rate of ripening changes, and whether or not ripening occurred off the vine. The diversity exhibited for ripening habit by the wild tomato species implies differences in regulatory mechanisms.

ACKNOWLEDGMENTS

I would especially like to acknowledge the help and support of my major professors, Dr. Jon Fobes and Dr. Robert C. Herner who were always ready to assist in any way possible. I would also like to acknowledge the other members of my guidance committee, Dr. Andrew Hanson and Dr. David Dilley for their assistance and helpful suggestions.

I would like to thank Jim O'Connel for growing and caring for my plants and Marcia Browers for helping me to make crosses. A special note goes to Beverly Burley for helping me to prepare the manuscript. Finally, I would like to thank Peter Irwin for coming to the rescue so many times when the G.C.'s weren't working.

TABLE OF CONTENTS

CHAPTER I

RIPENING BEHAVIOR OF WILD, COLOR-FRUITED TOMATO SPECIES

CHAPTER II

RIPENING BEHAVIOR OF WILD, GREEN-FRUITED TOMATO SPECIES: FRUITS THAT ABSCISE PRIOR TO RIPENING

CHAPTER III

RIPENING BEHAVIOR OF WILD, GREEN-FRUITED TOMATO SPECIES: FRUITS THAT RIPEN ON THE VINE

LIST OF TABLES

Table Page

CHAPTER I

CHAPTER II

CHAPTER III

LIST OF FIGURES

Figure | Page

CHAPTER I

CHAPTER II

CHAPTER III

Readers:

The paper format utilized in this thesis meets the requirments stipulated by the Horticulture Department and the University. The thesis body was separated into three sections. They were prepared for *Plant Physiology* and follow the manuscript style of that journal.

INTRODUCTION

The cultivated tomato, _L_. _esculentum_, has long been a choice experimental unit for investigations into the ripening process. Much progress has been made toward understanding the highly regulated series of biochemical and physiological changes that mark transition of an immature green tomato fruit, to a ripe tomato. These changes include bursts in ethylene production and respiratory activity, chlorophyll degradation, biosynthesis of flavor components and carotenoid pigments, breakdown of starches and fruit softening (9).

Early studies of respiratory activity in ripening fruits led to the discovery of the respiratory climacteric, an increase in respiration accompanying visible ripening changes (10). At approximately the same time, exogenous ethylene was reported to hasten ripening in a wide variety of fruits (1). Investigations into the role of endogenous ethylene production were thus stimulated (7). With the advent of gas chromatography, a rise in endogenous ethylene production was found to shortly preceed the rise in respiratory activity (3). Thus, a causal relationship between ethylene production and subsequent fruit ripening with its associated respiratory climateric was hypothesized. Other supportive evidence included the inhibition of ripening by removal of ethylene from fruit storage containers (1,4).

Endogenous ethylene production by ripening fruit is not a universal phenomenon, however. Other fruits were studied which produce very little ethylene and do not undergo a respiratory climacteric (13). These fruits have been termed non-climacteric. In addition, aspects of ripening in climacteric fruit suggest ethylene is not the sole regulator of ripening. There are examples of increased respiration occurring without increased ethylene production (20), and increased ethylene production occurring without heightened respiration (1).

Perhaps one of the strongest arguments for the limited role of ethylene in tomato is that immature green fruits cannot be induced to ripen normally by exposure to exogenous ethylene (9,12,15). Thus, some other changes must occur in the maturing fruit that enable it to respond to ethylene and subsequently ripen. Even clearer evidence for this comes from the rin and nor non-ripening tomato mutants. They remain green and hard for months, produce little ethylene and do not undergo a respiratory climacteric (8,11). In response to exogenous ethylene, they appear typically non-climacteric and are not induced to ripen (8,11). Thus, their block to ripening must lie at some point prior to the ability to respond to ethylene.

This provides many questions of physiological and genetic import. How do gene products differ between mutant and normal fruits? How are developmental processes regulated? What roles do selective gene expression, environment, and physiological conditions play? Perhaps the most fundamental question facing biologists today is how gene expression is regulated. The tomato system may offer an effective approach to answer this question.

Many models for ripening changes in tomato fruit have been proposed. The presence of genetically altered forms have enabled investigators to test these models more fully. Changes in hormone levels (2,5,6), membrane permeability (17,19), and various enzyme activities (15,16,18) have been assayed in normal and mutant fruits. Genetic diversity is therefore a potentially powerful tool to investigate physiological processes and understand the integration of genetics and physiology in the regulation of development.

If diversity exists for ripening behavior, it may be possible to manipulate the process genetically. This study was initiated to survey ripening habit among wild tomato species. Wild tomato populations exhibit variability for a host of characteristics, thereby enabling breeders to increase yields, incorporate disease and insect resistance, increase stress tolerances, and heighten nutritional values (14).

Observation in natural habitats suggests that variability for ripening behavior was likely: wild tomato fruits differ with respect to external ripening changes including softening, pigmentation, and abscission. Accordingly, the characterization of ripening undertaken for these fruits included determination of indices of ripening, age at maturity, ethylene production and respiratory behavior. The information obtained has led to hypotheses for ripening control mechanisms and has suggested numerous additional experiments.

LITERATURE CITED

1. Abeles, F. B. 1973. Ethylene in plant biology. Academic Press, New York.

2. Abdel-Rahman, M., T. H. Thomas, G. J. Doss and L. Howell. 1975. Changes in endogenous plant hormones in cherry tomato fruits during development and maturation. Physiol. Plant. 34:39-43.

3. Burg, S. P. and E. A. Burg. 1968. Ethylene, plant senescence and abscission. Plant Physiol. 43:1503-1511.

4. Burg, S. P. and E. A. Burg. 1966. Fruit storage at subatmosperic pressures. Science. 153:314-315.

5. Dilley, D. R. 1969. Hormonal control of fruit ripening. HortSci. 4:111-114.

6. Frenkel, C. and S. A. Garrison. 1976. Initiation of lycopene synthesis in the tomato mutant rin as influenced by oxygen and ethylene interactions. HortSci. 11:20-21.

7. Gane, R. 1935. The formation of ethylene by plant tissue and its significance in the ripening of fruits. J. Pomol. 18:351.

8. Herner, R. C. and K. C. Sink. 1973. Ethylene production and respiratory behavior of the rin tomato mutant. Plant Physiol. 52:38-42.

9. Hobson, G. E. and J. N. Davies. 1971. The tomato. In A. C. Hulme (ed.) Biochemistry of fruits and their products. Vol. 2. Academic Press, New York.

10. Kidd, F. and C. West. 1925. Gt. Brit., Dep. Sci. Ind. Res. Food Invest. Bd. Rep. 1924:27.

11. Ng, T. J. and E. G. Tigchelaar. 1977. Action of the non-ripening (nor) mutant on fruit ripening of tomato. J. Amer. Soc. Hort. Sci. 102:505-509.

12. Pratt, H. K. and J. D. Goeschl. 1969. Physiological role of ethylene in plants. Ann. Rev. Plant Physiol. 20:541-584.

13. Rhodes, M. J. C. 1970. The climacteric and ripening of fruits. In A. C. Hulme (ed.) Biochemistry of fruits and their products. Vol. 1. Academic Press, New York.

14. Rick, C. M. 1973. Potential genetic resources in tomato species: clued from observations in native habitats. In A. M. Syrb (ed.) Genes, enzymes and populations. Plenum Press, New York.

15. Sacher, J. A. 1973. Senescence and post-harvest biology. Ann. Rev. Plant Physiol. 24:197-224.

16. Sawamura, M., E. Knegt, and J. Bruinsma. 1978. Levels of endogenous ethylene, CO_2 and soluable pectin and activities of pectin methylesterase and polygalacturonase in ripening tomato fruits. Plant Cell Physiol. 19: 1061-1069.

17. Simon, E. W. 1974. Phospholipids and plant membrane permeability. New Phytol. 73:377-420.

18. Tigchelaar, E. C., W. B. McGlasson and R. W. Buescher. 1978. Genetic regulation of tomato fruit ripening. HortSci. 13:508-513.

19. Vickery, R. S. and J. Bruinsma. 1973. Compartments and permeability for potassium in developing fruits of tomato. J. Exp. Bot. 24:1261-1270.

20. Watada, A. and L. L. Morris. 1967. Growth and respiration patterns of snap bean fruits. Plant Physiol. 42:751-761.

CHAPTER I: RIPENING BEHAVIOR OF WILD, COLOR-FRUITED TOMATO SPECIES

ABSTRACT

Wild, color-fruited tomatoes, L. esculentum var. cerasiforme, L. pimpinellifolium, and L. cheesemanii, were surveyed for variability in ripening habit. Ethylene production and respiration of ripening fruits were monitored using an ethylene-free flow-through system and gas chromotography. The color-fruited tomatoes all exhibited climacteric ethylene production and respiratory behavior similar to the cultivated tomato. There were differences, however, for the timing and magnitude of climacteric response. Levels of ethylene production and CO_2 evolution were correlated with age at maturity over a three-fold range from the earliest maturing L. pimpinellofolium, to the latest maturing, L. cheesemanii, fruit.

INTRODUCTION

Ripening in the cultivated tomato, Lycopersicon esculentum, comprises a series of biochemical and physiological events, including the following: softening, pigment changes, development of flavor components, autocatalytic ethylene production and climacteric respiratory behavior (8). Many investigations have been made to elucidate the mechanisms initiating and integrating these processes, but much is unresolved.

Most workers agree that ethylene plays a central role in triggering climacteric fruit ripening (1,10,15,16,24). Mature green tomato fruits can be induced to ripen with exogenous ethylene; once ripening is initiated, there is an autocatalytic burst in ethylene production resulting in quantities far in excess of the threshhold level (8).

Ethylene, however, is not the sole regulator of ripening. Immature tomato fruits cannot be induced to ripen normally by treatment with ethylene (8,15,24). Thus, changes must first occur within the fruit before normal ripening can take place. Various possibilities that have been investigated include: changes in membrane permeability (26,29), changes in levels of endogenous hormones (2,3,4), and changes in the activities of various enzymes (24,25,28). There is not sufficient evidence, however, to establish any one of these as the key to ripening. There must be some as yet unresolved change, or series of changes, in the developing fruit which eventually enables response to ethylene and subsequent ripening.

The most basic resource that can be used for plant improvement is the genetic diversity available in natural populations of the cultigens and related species. Modern tomato breeding provides an excellent example of the exploitation of wild species by introgression to improve cultivated materials; disease- and pest-resistant cultivars are prime examples of breeders' successes (17). Within the gene pools of tomato species, much variability exists for various morphological (17) and biochemical characters (18, 20-23) and observations in wild habitats have suggested that considerable variation for ripening behavior is readily available for use in physiological studies and crop improvement. The nine known tomato species fall into two categories, color-fruited species and green-fruited species. The color-fruited species include Lycopersicon esculentum (the cultivated tomato), L. esculentum var. cerasiforme (the wild cherry tomato), L. pimpinellifolium, and L. cheesmanii. The latter three are readily crossable with the cultivated tomato. L. esculentum var. cerasiforme is a pantropic weed native to the Andean region, and also found in Brazil, Columbia, Central America, and Mexico. L. pimpinellifolium represents a diminutive esculentum, and is indigenous to the coastal zones of Peru and Ecuador; L. cheesmanii is a wild species endemic to the Galapagos Islands.

Genetic manipulation is only possible if there is variability among the materials. This study is a survey to characterize the wild tomato fruits on the basis of: 1) the type of visible ripening changes that occur, 2) age at maturity, 3) ethylene production, and 4) respiratory behavior.

MATERIALS AND METHODS

Plant materials: Wild, color-fruited tomato species were studied for ripening behavior and compared to the cultivated tomato. They are pictured by Figure 1 and the source of wild germplasm is listed in Table I. Only one accession of each species was tested. Flowers were selfed by hand so exact fruit age could be determined.

The plants were grown in an air-conditioned greenhouse (25/17°C day/night temperature), with 16 hours/day of supplemental lighting provided by 1000 watt metal halide lamps. Standard Michigan greenhouse cultural practices were followed.

Measurement of ethylene and carbon dioxide: To determine age at maturity for each species, 2-3 fruits were harvested every two or three days from the time they reached full size, until they began to change color. The resultant set of 10 of 12 fruits was monitored for ethylene production and CO_2 evolution. Once age at maturity was determined, another set of 10 to 12 fruits was harvested just prior to the onset of ripening and monitored for respiration and ethylene production.

Detached fruits were weighed, placed individually into 50 ml. jars containing a layer of glass beads, and held in a 20°C storage room with 16 hours of mixed incandescent and flourescent light per day. The jars were connected to a calibrated, humidified, ethylene-free flow-through system.

Figure 1: Wild, color-fruited tomatoes. Left to right: *Lycopersicon esculentum* var. *cerasiforme*, *L. pimpinellifolium*, *L. cheesmanii*.

Table I. Tomato Germplasm Used For Assay of Ripening Characteristics.

LA. No.	Species	Location
490	*Lycopersicon esculentum*, cv. VF36	
1268	*L. esculentum* var. *cerasiforme*	Chaclacayo, Lima, Perú
1633	*L. pimpinellifolium*	Chinca, Ica, Perú
1400	*L. cheesmanii*	Isabella, Galapagos Islands, Ecuador

The fruits weighed 2 g. or less (Table II), so very slow flow rates of 1-2 ml. min.$^{-1}$ were used.

Two, one ml. samples were taken from the effluent air stream each day at the same time and analyzed for C_2H_4 and CO_2 by gas chromatography. Ethylene production was expressed as µl C_2H_4 kg. fresh wt.$^{-1}$ day^{-1}; CO_2 evolution as mg. CO_2 kg. fresh wt.$^{-1}$ day^{-1}.

Propylene studies: Propylene, an ethylene analouge, can be used to simulate the effects of exogenous ethylene on fruit ripening (13). L. pimpinellifolium fruit were harvested approximately eight days prior to the onset of ripening and placed individually into sealed liter containers. Propylene was added to give a final concentration of 1000 ppm.; an equivilent volume of air was added to the control containers. Once a day, the fruits were removed to 50 ml. jars and capped. Two, 1 ml gas samples were taken from these jars one and two hours after capping and analyzed for CO_2 and ethylene.

RESULTS

The four tomato species exhibited variability for ripening characteristics, including the following; age at maturity, ethylene production, respiratory behavior and pigment composition. Quantitative evaluation of these characters is described below.

The indicator of ripeness used for maturity determinations was color change -- red coloration in VF36, L. cerasiforme, and L. pimpinellifolium, and orange coloration in L. cheesmanii;

thus age of maturity was easily determined (Table II). For the green fruited species, it was more difficult to distinguish "ripening signals" and age at maturity.

Figure 2 illustrates the relationship between the age of fruit when harvested, and days until fully ripe for the color-fruited species. There is a negative 1:1 linear relationship indicating that ripening of mature fruits occurs at the same rate on, or off, the vine. If the fruits are harvested too young, however, they never attain full color.

Ethylene biosynthesis: Fruits are generally classified into one of two categories, climacteric or non-climacteric, based on their patterns of ethylene biosynthesis and respiratory activity (16). The cultivated tomato is classified as a climacteric fruit (16); it shows a peak in ethylene production and respiratory activity concomitant with ripening. Figure 3a illustrates ethylene production for the three wild, color-fruited tomato species. All three have typical climacteric ethylene production; however, there are differences in timing and magnitude of peak ethylene production among the species.

Peak ethylene production for L. pimpinellifolium is at 37 days, L. esculentum var. cerasiforme at 42 days, and L. cheesmanii at 47 days. It is interesting to note that as time required to reach the climacteric peak increases, the level of ethylene production increases. The earliest maturing species,

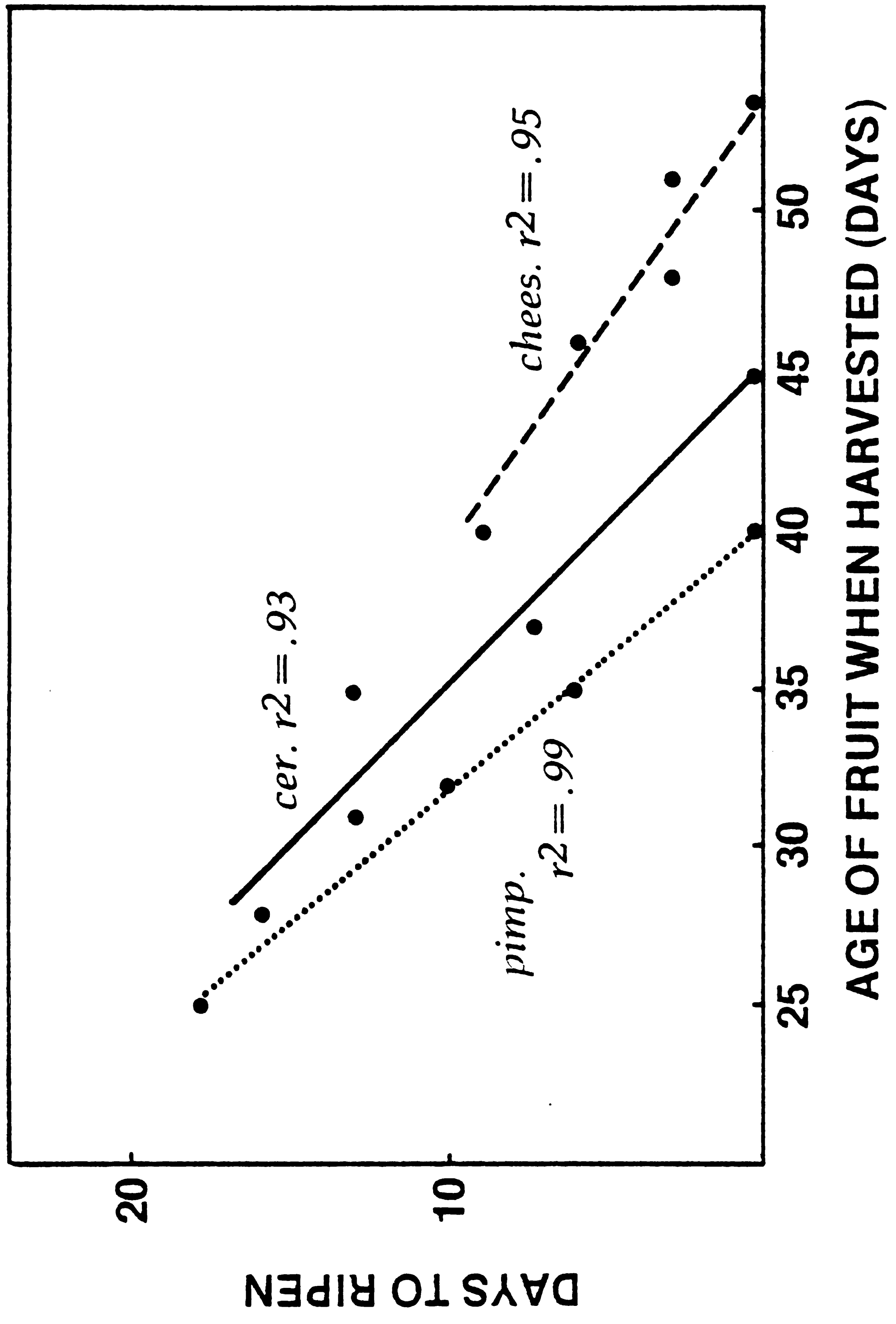
Table II. Fruit Weight and Approximate Age at Maturity: Color-fruited Tomato Species.

Species	Fresh weight (g)[1]	Age (days after pollination)
Lycopersicon esculentum cv. VF36	>40	40
L. esculentum var. *cerasiforme*	2.04 ± 0.26	45
L. pimpinellifolium	0.74 ± 0.05	40
L. cheesmanii	0.19 ± 0.04	55

---

[1] mean of 10 fruits ± SE

Figure 2: Days to ripen vs. age at harvest for the wild, color-fruited tomatoes.


DAYS TO RIPEN
20
10
AGE OF FRUIT WHEN HARVESTED (DAYS)
25
30
35
40
45
50
cer. r2 = .93
pimp. r2 = .99
chees. r2 = .95

L. pimpinellifolium, has peak ethylene production of 75-150 $\mu l\ kg^{-1}\ day^{-1}$; the latest maturing species, *L. cheesmanii*, has peak production at least three times higher (Figure 3a, Table III). Ethylene production in *L. esculentum* var. *cerasiforme* is very similar to production in the cultivated tomato.

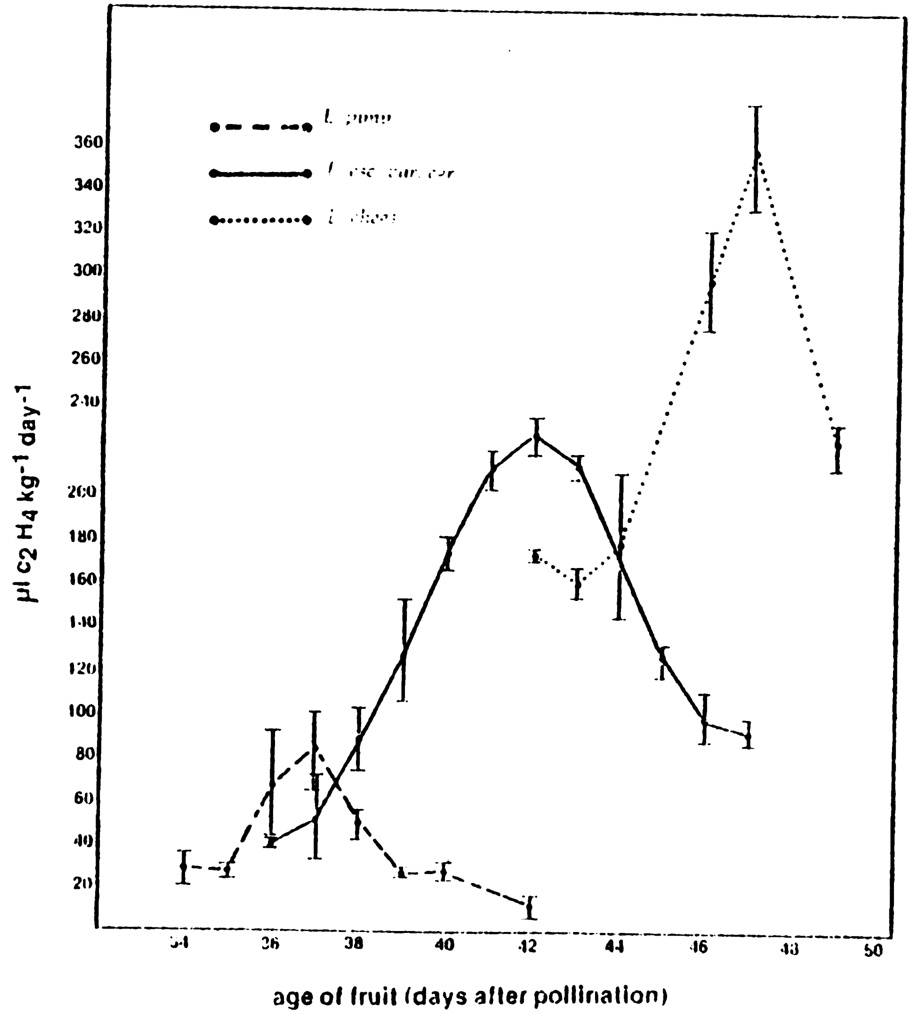
Respiratory behavior: Figure 3b illustrates the relationship of CO_2 evolution to fruit age in the color-fruited species. *L. esculentum* var. *cerasiforme* and *L. cheesmanii* exhibit climacteric respiratory behavior reaching peak CO_2 production at 42 and 47 days respectively. *L. pimpinellifolium*, however, had a steady, low level of respiration more characteristic of non-climacteric fruits (16).

When three *L. pimpinellifolium* fruits, rather than one fruit, were put together in a jar, a small respiratory climacteric was detected (Fig. 4). This suggests that the rate of respiration is too low to distinguish a climacteric rise in individual fruits. The time scale for ripening has been shifted in this experiment, because the fruits were grown in the winter. Fruits used for Figures 2 and 3 were grown in the summer.

As was true for peak ethylene production, the later maturing fruits have considerably higher respiratory activity (Table III). The earliest maturing species, *L. pimpinellifolium*, had peak CO_2 production of 800-1000 mg. $CO_2\ kg^{-1}$

Figure 3: Ethylene production (a) and respiratory behavior (b) during ripening in the wild, color-fruited tomatoes: L. esculentum var. cerasiforme, L. pimpinellifolium and L. cheesmanii.[1] Full color development was attained 3-5 days after peak ethylene production.

[1] Data are the means of 8, 5 and 3 replicates respectively ± S.E.


L. pimp
L. esc. var. cer
L. chees
μl C2 H4 kg-1 day-1
360
340
320
300
280
260
240
200
180
160
140
120
100
80
60
40
20
34
36
38
40
42
44
46
48
50
age of fruit (days after pollination)

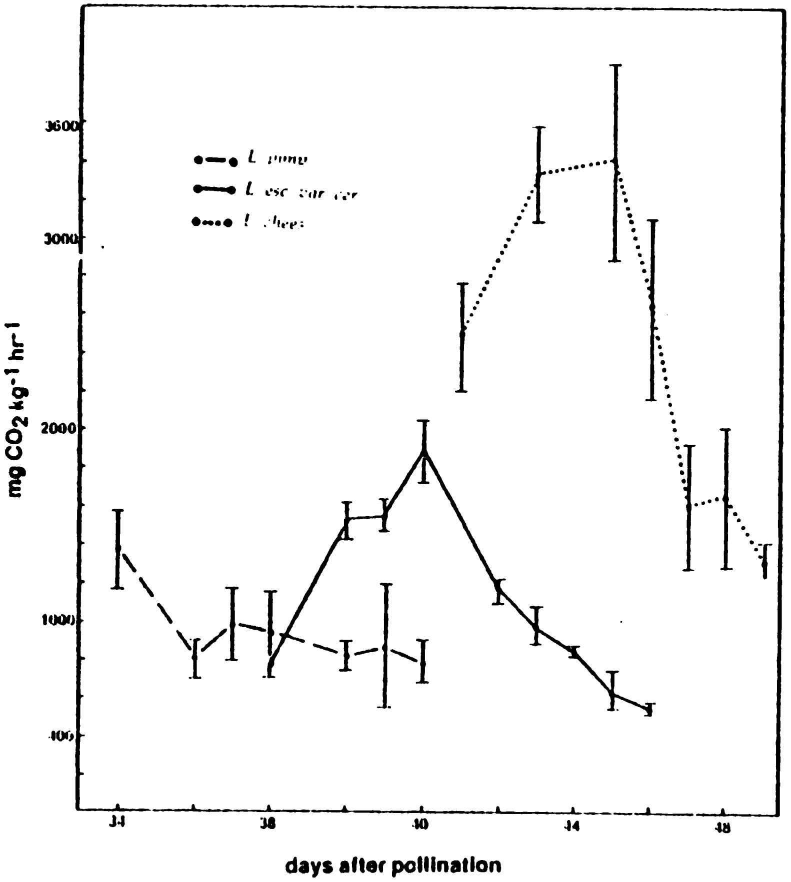

L. pimp
L. esc. var. cer
L. chees
mg CO2 kg-1 hr-1
3600
3000
2000
1000
400
34
38
40
44
48
days after pollination

Figure 4: Ethylene production and respiratory activity for three _L_. _pimpinellifolium_ fruit per jar.

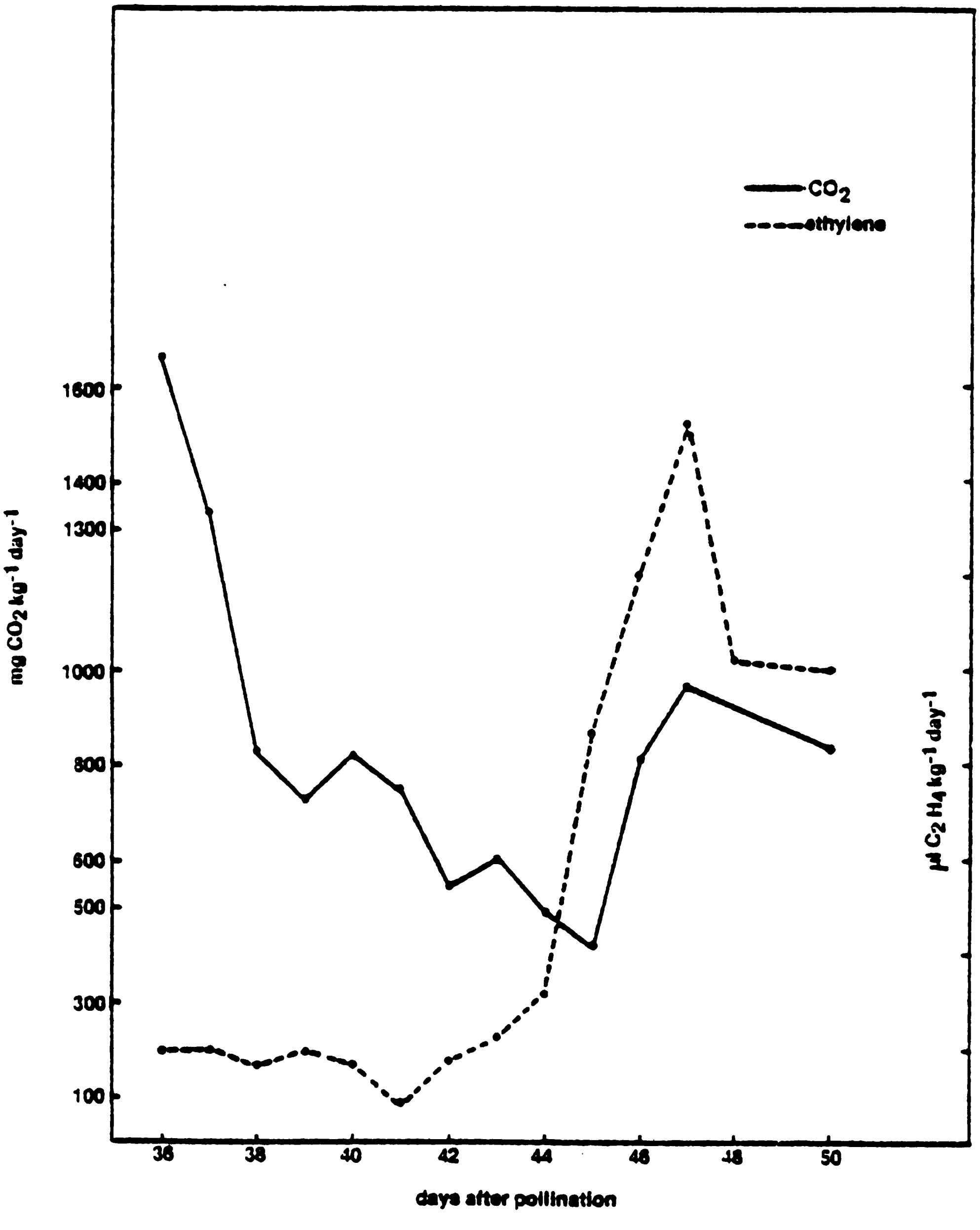

CO2
ethylene
mg CO2 kg-1 day-1
1600
1400
1300
1000
800
600
500
300
100
µl C2 H4 kg-1 day-1
160
140
120
100
80
60
40
20
36
38
40
42
44
46
48
50
days after pollination

Table III. Comparison of Peak Ethylene Production and CO_2 Evolution during Ripening in the Wild, Color-Fruited Tomato Species. Summary of several experiments.

Species	Ethylene Production ($\mu l\ kg^{-1}\ day^{-1}$)	CO_2 Evolution ($mg\ kg^{-1}\ day^{-1}$)
Lycopersicon pimpinellifolium	75-150	800-1000
L. esculentum var. *cerasiforme*	200-250	1000-2000
L. esculentum cv. VF 36	200-250	1000-2000
L. cheesmanii	300-400	3000-4000

day^{-1}, while L. cheesmanii exhibited peak CO_2 evolution of 3000-4000 mg. CO_2 kg^{-1} day^{-1}.

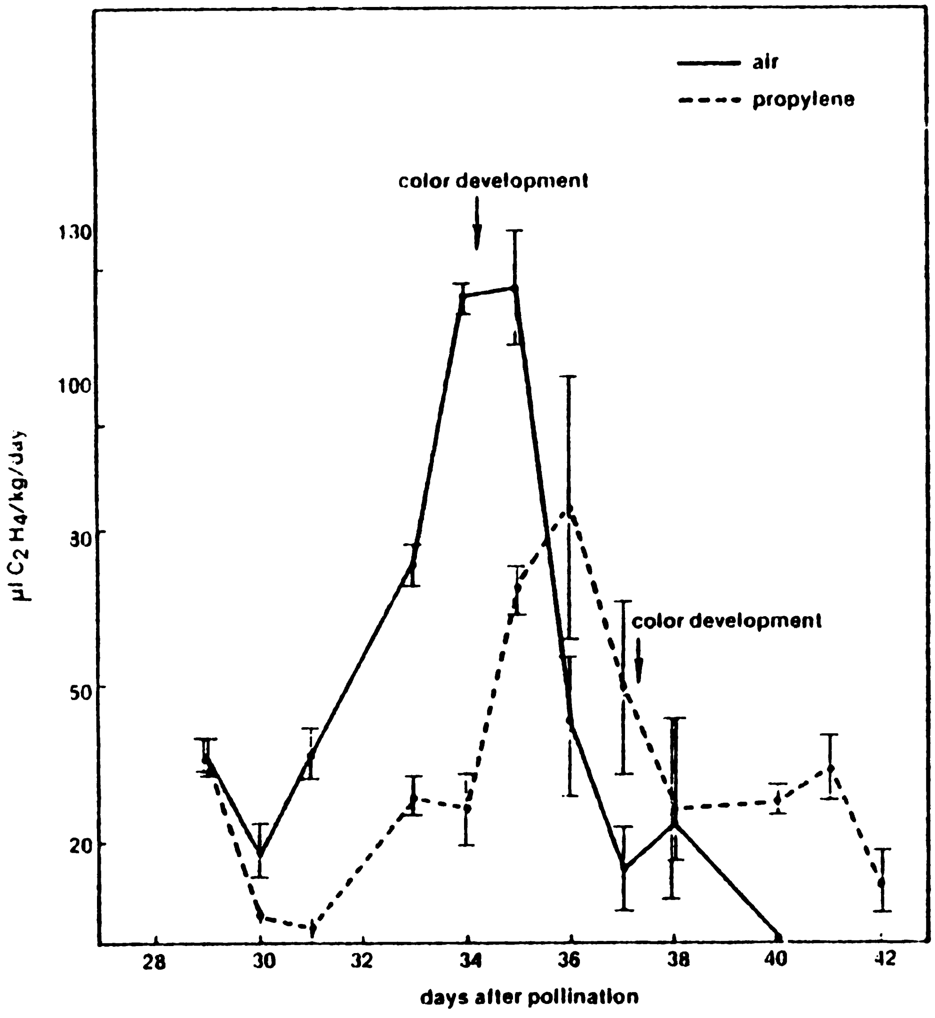
Response to propylene treatment: Another characteristic of climacteric fruit behavior is hastened ethylene production, increased respiration, and earlier ripening in response to propylene treatment (13). Since the respiratory trend for L. pimpinellifolium was unclear, fruits of this species were treated with propylene. The resultant ethylene production is illustrated by Figure 5a. Propylene treatment hastens endogenous ethylene production, and color development occurred three days earlier for propylene treated fruit than for control fruit. Respiration of treated and control fruits stored individually declined to low, sustained levels (Figure 5b). There was no discernable difference between the two groups.

DISCUSSION

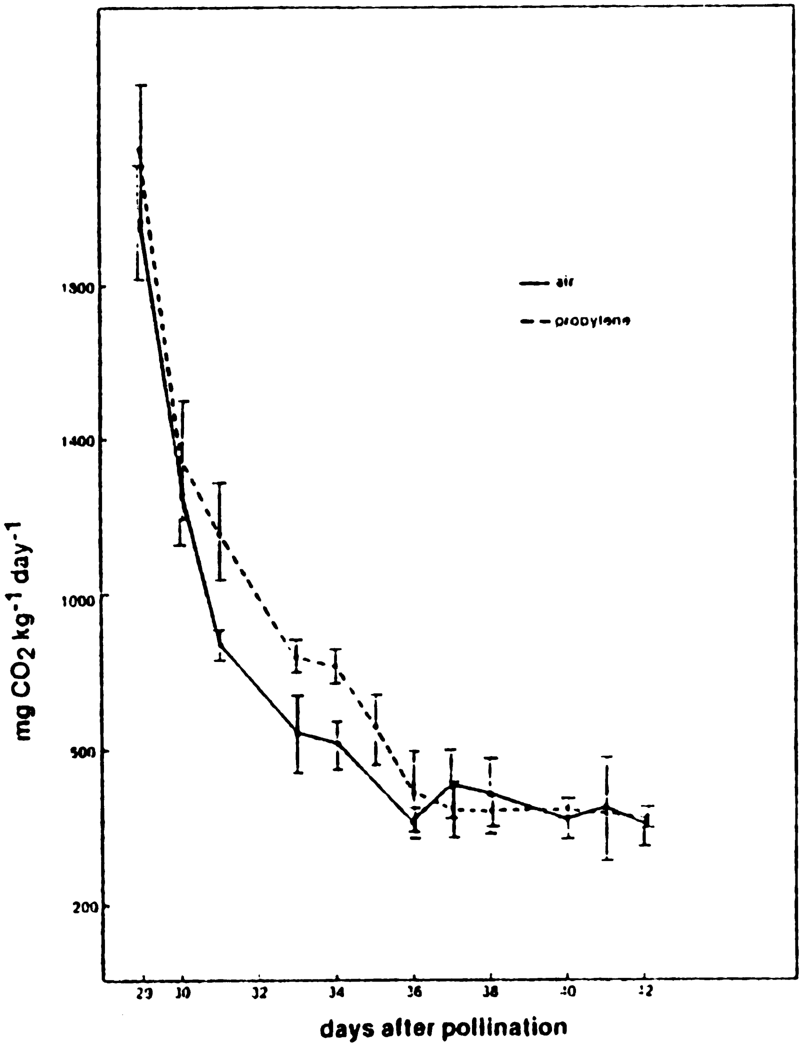
Variability for ripening characteristics among the wild tomato species represents a genetic pool for future physiological investigations and for possible incorporation of specific control mechanisms into the ripening process of cultivated tomatoes. The color-fruited species are predicatably, quite close in ripening behavior to the cultivated tomato. In addition to chlorophyll degradation, carotenoid biosynthesis, and softening, the three color-fruited species

Figure 5: The effect of propylene treatment on ethylene production (a), respiration (b) and color development of L. pimpinellifolium fruit.[1]

[1] Data are the mean of 3 replicates ± S.E.



1) arrows indicate first noticable red color



all show fairly typical climacteric behavior. They all exhibit a peak in ethylene production that is correlated with other ripening phenomena such as softening, color change, and climacteric respiration (Figure 3). Peak ethylene production preceeds full color development in these fruits by three to five days, as is characteristic of the cultivated tomato (16).

The wild color-fruited species have different magnitudes of ethylene production directly correlated to their age at maturity. The older fruits produce higher amounts of ethylene. Those fruits that mature later also have higher base levels of ethylene production prior to the climacteric burst.

A poorly understood phase in fruit ripening is the transition from the immature pre-climacteric fruit to the mature pre-climacteric fruit. In tomatoes the basic ethylene forming mechanism exists at a low level throughout development, but it is not fully activated until a critical physiological age (13). If fruit are harvested too young, they are never capable of being activated (15). Thus, some sort of change must occur during the transition from an immature to mature fruit. The change might be a gradual increase in sensitivity to endogenous ethylene, a gradual increase in endogenous ethylene to a threshhold concentration (11), a loss of feedback inhibition (15), or a combination of these possibilities.

McGlasson *et al*. (11) studied the response of immature tomato fruits to propylene treatment. Their results show that the ability of immature fruits to respond to propylene increases with fruit age. They suggested that some relatively slow change must preceed the burst in ethylene production and that the development of some cellular component during aging may result in a system capable of autocatalytic ethylene production. Our results support this hypothesis. Since the base level of ethylene production prior to the climacteric is lower in earlier maturing fruits, these fruits may have a lower threshhold level for response. If the threshhold barrier for autocatalytic ethylene production and ripening is broken sooner, perhaps the cellular component developed during fruit aging that enables autocatalytic ethylene production is not developed to the same extent as in later maturing fruits. Thus the capacity for autocatalytic ethylene production would be lower in earlier maturing fruits.

An interesting next experiment would be to test for threshhold level of response to propylene treatment in fruits of the different species. One would hypothesize that *L. pimpinellifolium* would require a lower threshhold concentration than *L. cheesmanii*.

Although the same kind of comparison relating age at maturity and levels of ethylene production cannot be made for different cultivars within *L. esculentum* (7,14,28), it is not contradictory to this speculation. Through centuries of domestication man has manipulated the cultivated tomato to suit his needs, including selection for uniform, early ripening (19). Thus it is not unreasonable to presume that the development of early and late cultivars has altered the relationship between age at maturity and capacity for ethylene production.

The negative linear relationship between age at harvest, and days to ripen, for the color-fruited species is consistent with ripening behavior in the cultivated tomato (9). Once of sufficient maturity, the machinery is present, and ripening will proceed on or off the vine at approximately the same rate (12). This is not the case for several of the green-fruited tomatoes (5,6). As is the case of the cultivated tomato, it is possible to harvest the wild, color-fruited tomatoes while still immature (16). These fruits never ripen fully after being removed from the vine.

Thus, ripening behavior in the color-fruited tomato species is closely related to ripening in the cultivated tomato. These fruits exhibit typical climacteric ethylene production and respiratory behavior, followed by softening and pigment changes. There is a difference among the species for timing of the ripening process and magnitude of the climacteric response. The direct relationship between fruit age at maturity, and magnitude of autocatalytic ethylene production suggests that some factor which promotes ripening by enabling autocatalytic production, increases with fruit age.

LITERATURE CITED

1. Abeles, F. B. 1973. Ethylene in plant biology. Academic Press, New York.

2. Abdel-Rahman, M., T. H. Thomas, G. J. Doss and L. Howell. 1975. Changes in endogenous plant hormones in cherry tomato fruits during development and maturation. Physiol. Plant. 34:39-43.

3. Dilley, D. R. 1969. Hormonal control of fruit ripening. HortSci. 4:111-114.

4. Frenkel, C. and S. A. Garrison. 1976. Initiation of lycopene synthesis in the tomato mutant <u>rin</u> as influenced by oxygen and ethylene interactions. HortSci. 11:20-21.

5. Grumet, R., J. F. Fobes and R. C. Herner. Ripening behavior in wild, green fruited tomato species: fruits that abscise prior to ripening. (in preparation).

6. Grumet, R., J. F. Fobes and R. C. Herner. Ripening behavior in wild, green fruited tomato species: fruits that ripen on the vine. (master's thesis).

7. Herner, R. C. and K. C. Sink. 1973. Ethylene production and respiratory behavior of the <u>rin</u> tomato mutant. Plant Physiol. 52:38-42.

8. Hobson, G. E. and J. N. Davies. 1971. The tomato. <u>In</u> A. C. Hulme (ed.) Biochemistry of fruits and their products, Vol. 2. Academic Press, New York.

9. Kader, A. A., M. A. Stevens, M. Albright-Holton, L. L. Morris and M. Algazi. 1977. Effect of fruit ripeness when picked on flavor and composition in fresh market tomatoes. J. Amer. Soc. Hort. Sci. 102:724-731.

10. McGlasson, W. B. 1970. The ethylene factor. <u>In</u> A. C. Hulme (ed.) Biochemistry of fruits and their products, Vol. 2. Academic Press, New York.

11. McGlasson, W. B., H. C. Dostal, and E. C. Tigchelaar. 1975. Comparison of propylene induced responses of immature normal and <u>rin</u> mutant tomatoes. Plant Physiol. 55:218-222.

12. McGlasson, W. B. and I. Adato. 1977. Relationship between the capacity to ripen and ontogeny in tomato fruits. Aust. J. Plant. Physiol. 4:451-458.

13. McMurchie, E. J., W. B. McGlasson and I. L. Eaks. 1972. Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237:235-236.

14. Ng, T. J. and E. G. Tigchelaar. 1977. Action of the non-ripening (<u>nor</u>) mutant on fruit ripening of tomato. J. Amer. Soc. Hort. Sci. 102:505-509.

15. Pratt, H. K. and J. D. Geoschl. 1969. Physiological role of ethylene in plants. Ann. Rev. Plant Physiol. 20:541-584.

16. Rhodes, M. J. C. 1970. The climacteric and ripening of fruits. <u>In</u> A. C. Hulme (ed.) Biochemistry of fruits and their products, Vol. 1. Academic Press, New York.

17. Rick, C. M. 1973. Potential genetic resources in tomato species: clued from observation in native habitats. <u>In</u> A. M. Srb (ed.) Genes enzymes, and populations. Plenum Press, New York.

18. Rick, C. M. 1975. The tomato. <u>In</u> R. C. King (ed.) Handbook of genetics, Vol. 2. Plenum Press, New York.

19. Rick, C. M. 1978. The tomato. Sci. Amer. 239:76-87.

20. Rick, C. M. and J. F. Fobes. 1975. Allozymes of Galapagos tomatoes: polymorphism, geographic distribution and affinities. Evolution. 29:443-457.

21. Rick C. M. and J. F. Fobes. 1975. Allozyme variation in the cultivated tomato and closely related species. Bull. Torrey Bot. Club 102:376-384.

22. Rick, C. M., J. F. Fobes and M. Holle. 1977. Genetic variation in <u>Lycopersicon</u> <u>pimpinellifolium</u>: evidence of evolutionary change in mating systems. Plant System. and Evol. 127:139-170.

23. Rick, C. M. and E. Kesicki, J. F. Fobes and M. Holle. 1976. Genetic and biosystematic studies on two new sibling species of <u>Lycopersicon</u> from interandean Peru.

24. Sacher, J. A. 1973. Senescence and post-harvest biology. Ann. Rev. Plant Physiol. 24:197-224.

25. Sawamura, M., E. Knegt and J. Bruinsma. 1978. Levels of endogenous ethylene, CO_2 and soluable pectin and activities of pectin methylesterase and polygalacturonase in ripening tomato fruits. Plant Cell Physiol. 19:1061-1069.

26. Simon, E. W. 1974. Phospholipids and plant membrane permeability. New Phytol. 73:337-420.

27. Tigchelaar, E. C., W. B. McGlasson and R. W. Buescher. 1978. Genetic regulation of tomato fruit ripening. HortSci. 13:508-513.

28. Tigchelaar, E. C., W. B. McGlasson and M. J. Franklin. 1978. Natural and ethephon stimulated ripening of F_1 hybrids of the ripening inhibitor (rin) and non-ripening (nor) mutants of tomato. Aust. J. Plant Physiol. 5:449-456.

29. Vickery, R. S. and Bruinsma, J. 1973. Compartments and permeability for potassium in developing fruits of tomato. J. Exp. Bot. 24:1261-1270.

CHAPTER II: RIPENING BEHAVIOR OF WILD, GREEN-FRUITED TOMATO SPECIES: FRUITS THAT ABSCISE PRIOR TO RIPENING.

ABSTRACT

Fruit of two wild, green-fruited tomato species, L. chilense and L. peruvianum were studied for ripening habit. These species are different from other tomatoes because their fruit abscise prior to ripening. Ethylene production and external ripening changes were studied in fruits collected prior to and following abscission. Ethylene production was monitored using an ethylene-free flow-through system and gas chromatography. The ability of fruits of the two species to produce ethylene and ripen varied with maturity. The two species differed from each other for timing of endogenous ethylene production relative to abscission. L. peruvianum fruit have elevated ethylene production preceding abscission. In L. chilense fruit, high levels of endogenous ethylene production only occurs after fruit drop. This suggests differences in control mechanisms regulating ripening in the two types of fruit.

INTRODUCTION

Many investigations have been made in recent years to elucidate the mechanisms initiating and integrating changes associated with tomato fruit ripening. Much, however, is unresolved. Most workers agree that ethylene plays a key role in triggering the process. In cultivated tomatoes and the closely allied color-fruited species ethylene production shortly precedes changes in color, texture, and flavor (7,10). Mature green fruits can be induced to ripen normally by exposure to exogenous ethylene (1,7). Furthermore, removal of ethylene by scrubbing the atmosphere or reduced atmospheric pressures inhibits ripening (1).

Ethylene, however, is not the only factor involved. Immature tomato fruits cannot be induced to ripen normally by treatment with ethylene (9,12,14). Thus, changes must occur within the fruit before ripening can take place. Various possibilities that have been investigated include: changes in membrane permeability (16,19), changes in levels of endogenous hormones (3,4,5), and changes in the activities if various enzymes (14,15,17). There is not sufficient evidence, however, to establish any one of these as the key to ripening. There must be some as yet unresolved change, or series of changes, in the developing fruit which eventually enables response to ethylene and subsequent ripening.

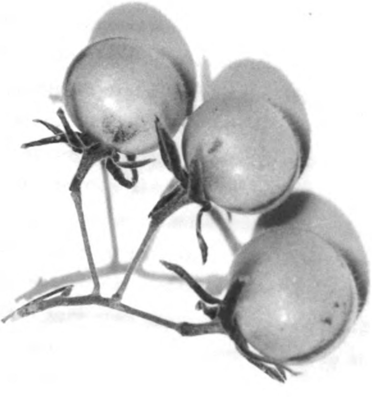
This study characterizes ripening in two wild, green-fruited tomato species, L. chilense and L. peruvianum. L. chilense is commonly found in the slopes and bottoms of quebradas that disect the desert of southern Peru and northern Chile; L. peruvianum inhabits multifarious niches in the coastal river valleys of Peru and northern Chile. Fruit from both species do not ripen until they have abscised from the plant. The relationship between ethylene production and the transition from immature, non-ripening, to mature, ripening fruit was investigated in these two species; the results may lead to a better understanding of control mechanisms underlying this transition.

MATERIALS AND METHODS

Two green-fruited, wild tomato species, *L. peruvianum* and *L. chilense*, were examined for ripening behavior. They are pictured by Figure 1. *L. peruvianum* was LA 1351 from Rope, Cajamarca, Peru; *L. chilense* was LA 455 from Tambo, Ariquipa, Peru. Only one accession of each species was tested. Since both species are self-incompatable, cross-pollinationswere made by hand; pollinated flowers were tagged so that exact fruit age could be determined. Growing conditions were the same as for Grumet, et al. (7).

Fruits harvested at various stages prior to abscission as well as fruits that naturally abscised from the plant were monitored for ethylene production. Ten to twelve fruits were used to characterize any one stage. For *L. chilense*, the stages delineated were: 1) prior to and during chlorophyll loss, 2) subsequent to chlorophyll loss, but prior to abscission, and 3) after abscission. For *L. peruvianum*, the stages studied were prior to and following abscission. Ethylene measurements were done as in Grumet, *et al*. (7). Propylene studies also followed the procedure of Grumet, *et al*. (7).

Figure 1: Green-fruited tomatoes that abscise prior to ripening: Left, *Lycopersicon chilense*; right *L. peruvianum*.



RESULTS

Indices of ripening in the wild tomato species include color change, softening and abscission (7,8). This paper describes two green-fruited species for which abscission is the first external indicator of ripening, _L_. _chilense_ and _L_. _peruvianum_.

_L_. _chilense_: _L_. _chilense_ fruits showed various patterns of ethylene production (Figures 2,3), depending on when they were collected. Natural abscission generally takes place after the fruits are 100 days old.

One of the normal developmental stages in _L_. _chilense_ fruit is a loss of chlorophyll beginning approximately 55 days after pollination. Fruits change from green to whitish; there is neither concomitant nor subsequent carotenoid biosynthesis. The loss of chlorophyll takes several days, as opposed to rapid degradation characteristic of the climacteric, color-fruited tomato species (7). It is also not accompanied by ethylene production (Table I).

Older fruits that had already lost chlorophyll, fell into two groups. Up until approximately eighty days, they produce almost no ethylene and do not respond to propylene by endogenous ethylene production (Figure 2a). When harvested more than eighty days after pollination, they produce small amounts of ethylene, up to 40 μl kg^{-1} day^{-1} (Figure 2b). If these fruits are treated with

Figure 2: Ethylene production of younger (a) and older (b) *L. chilense* fruit with, and without, propylene. Both sets of fruit had already lost their chlorophyll

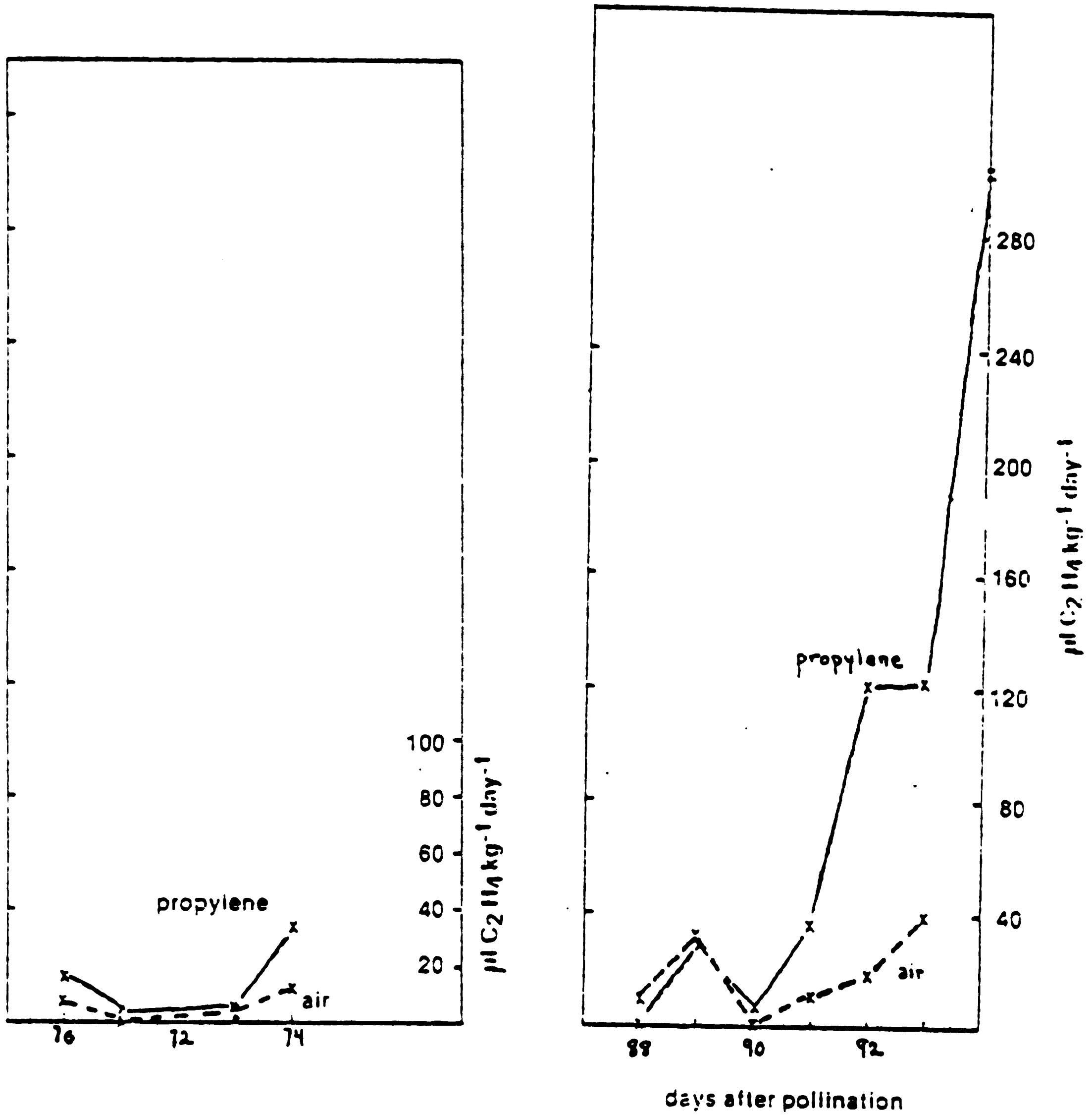


Figure 1: Ethylene production of younger (a) and older (b) *L. chilense* fruit with, and without, propylene. Both sets of fruit had already lost their chlorophyll.

Figure 3: Ethylene production in abscised _L_. _chilense_ fruit.

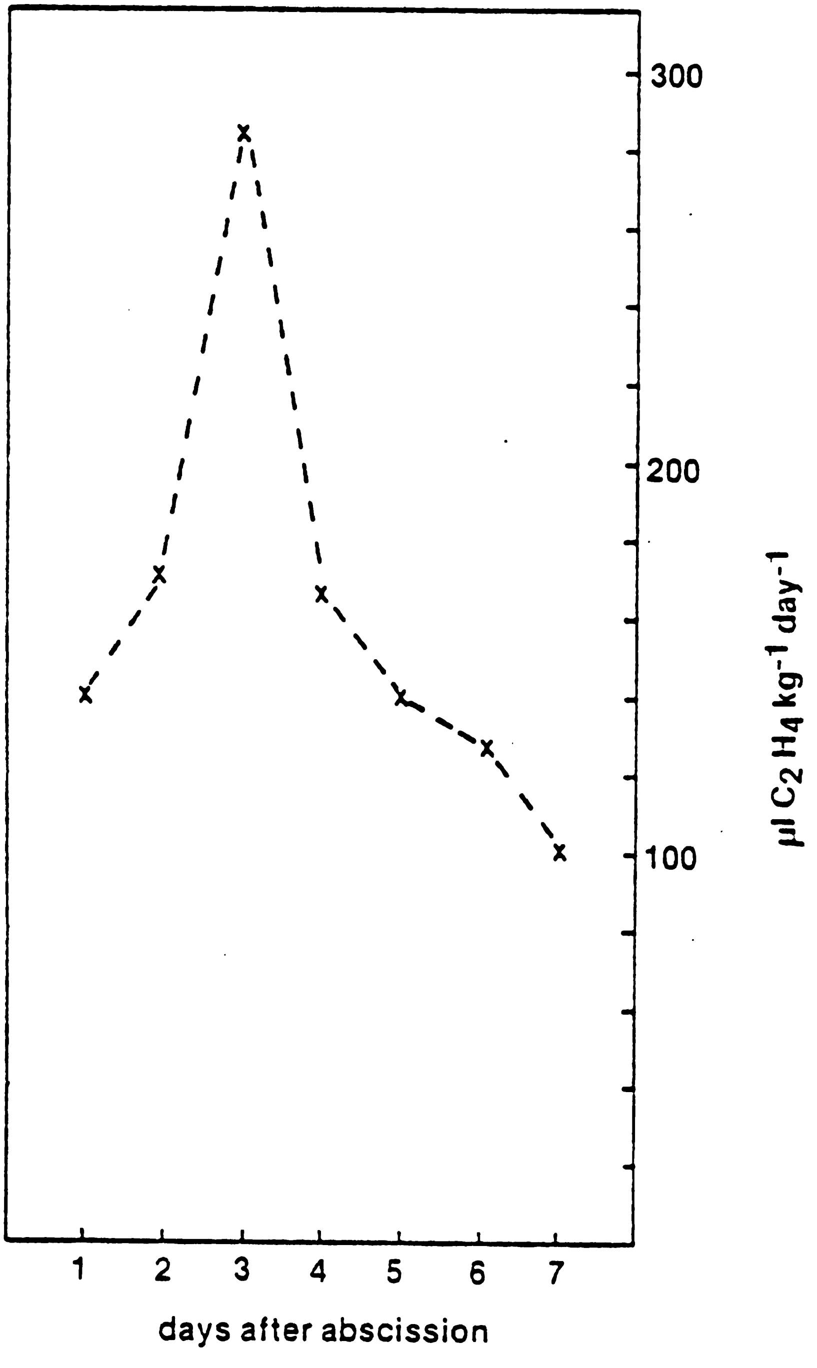

300
200
100
1
2
3
4
5
6
7
days after abscission
μl C2 H4 kg-1 day-1

Table I: Levels of Endogenous Ethylene Production in _L_. _chilense_ Fruit at Various Stages of Development - Summary of Several Experiments.

Age (days after pollination)	Stage of Development	Peak Ethylene Production (ul kg^{-1} day^{-1})
55-65	during chlorophyll loss	0
65-80	maturation	10
80-100	pre-abscission	40
100+	abscised	300

propylene, they are stimulated to produce as much as 300 µl C_2H_4 kg^{-1} day^{-1} (Figure 2b). Finally, fruits that naturally abscise exhibit a climacteric rise in ethylene production to 300 µl kg^{-1} day^{-1} (Figure 3).

L. peruvianum: The first external sign of ripeness in L. peruvianum fruit is abscission from the vine approximately 70 days after pollination. Fruits remain green and hard while on the vine and soften after they fall. There is no loss of chlorophyll corresponding to degreening of L. chilense fruit. L. peruvianum fruit remain green even after they abscise and soften.

Fruits harvested prior to natural abscission fall into two categories: a minority that softens, and the majority that does not. Fruits that do not soften have lower ethylene production that those that do (Figure 4). Hand picked fruits that do not soften can be further divided into two groups based on ease of detachment from the plant by breaking at the abscission zone. There is a difference in the type of break that occurs in the two groups of fruits. Those that are difficult to break leave a rough interface at the abscission zone. Those that are not as difficult to break leave a smooth interface. Fruits that break off relatively easily also have higher levels of ethylene production than those that are difficult to break (Table II). Both have lower ethylene production than fruits that soften.

Figure 4: Ethylene production in softening and non-softening, hand-detached *L. peruvianum* fruits. Softening occurred in the high ethylene producing group at 66 days.

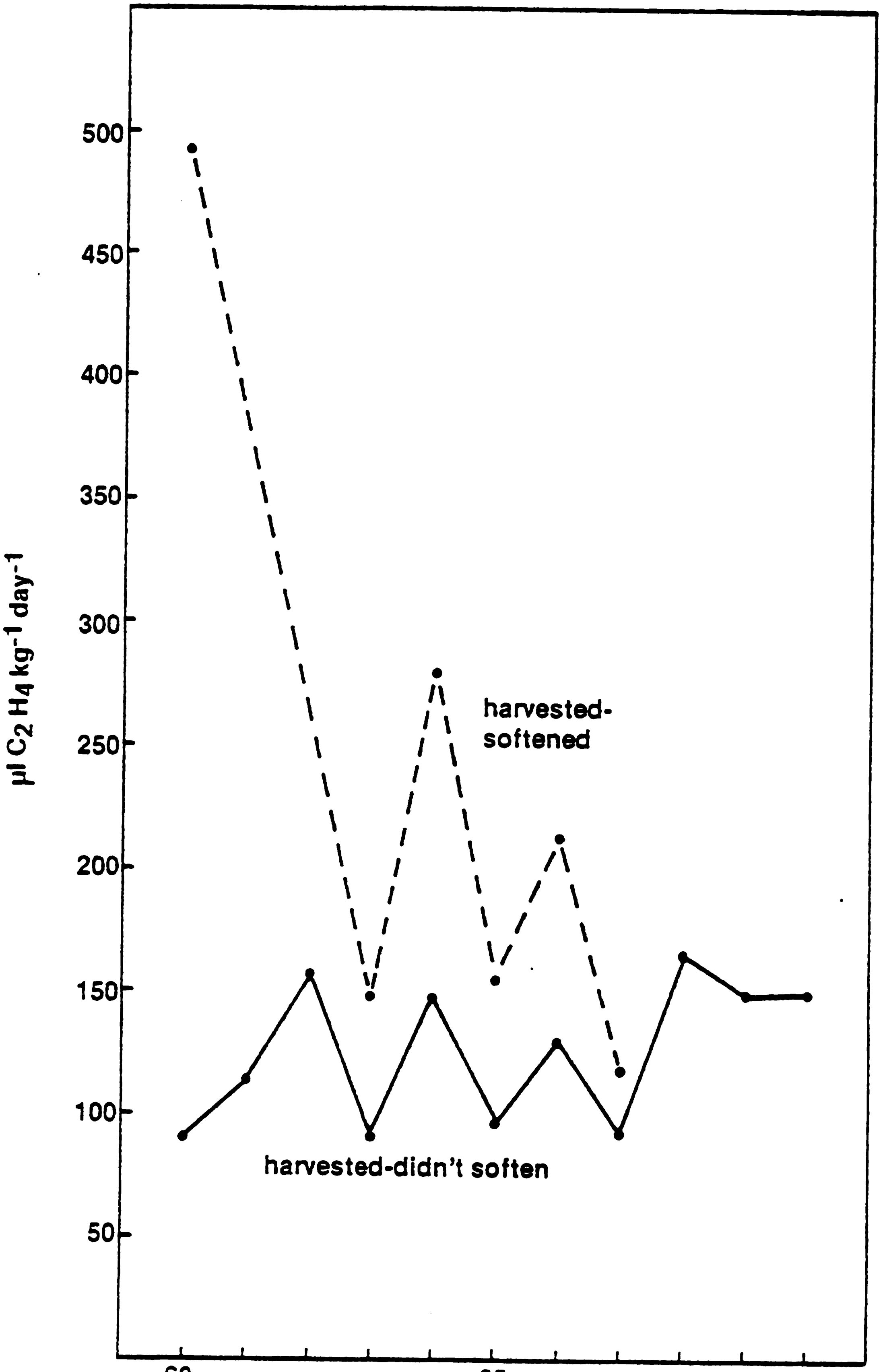

500
450
400
350
300
250
200
150
100
50
µl C_2H_4 kg^{-1} day^{-1}
harvested-softened
harvested-didn't soften
60
65
70
days after pollination

Table II. Peak Ethylene Production in Four Categories of *L. peruvianum* Fruit.

Stage of Development	Peak Ethylene Production ul kg^{-1} day^{-1}	Softened
hard to detach	80	no
not difficult to detach	160	no
about to fall	350	yes
fallen	550	yes

Another experiment was done to eliminate the possibility that wounding is responsible for high ethylene production in the fruits that soften. Since the most feasible source of wounding during hand-detachment is removal of the calyx, fruits were harvested and monitored with their calyxes attached. Figure 5 shows relative positions of the natural abscission zone and point of calyx attachment. After three days in storage, calyxes were removed from fruit with low initial ethylene production. The results show that calyx removal does not increase ethylene production or induce softening (unpublished data). Fruits having high initial levels of ethylene production were not included in the experiment because an increase in production due to calyx removal would not be detected. These fruits softened with their calyzes attached, as do fruits that naturally drop from the vine.

Fruits that fell from the vine were also studied and found to have high levels of ethylene production (Figure 6). They softened within a few days after abscission (Table I). Also studied were fruits that fell off the vine when they were touched. These fruits were estimated to be one day prior to abscission. They also soften quickly and have high levels of ethylene production (Figure 6).

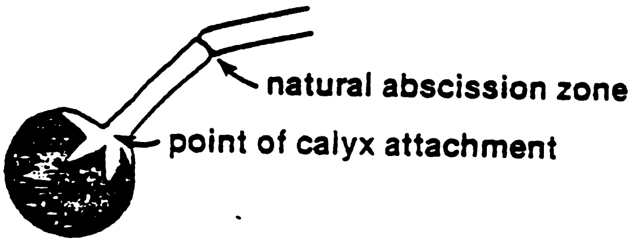
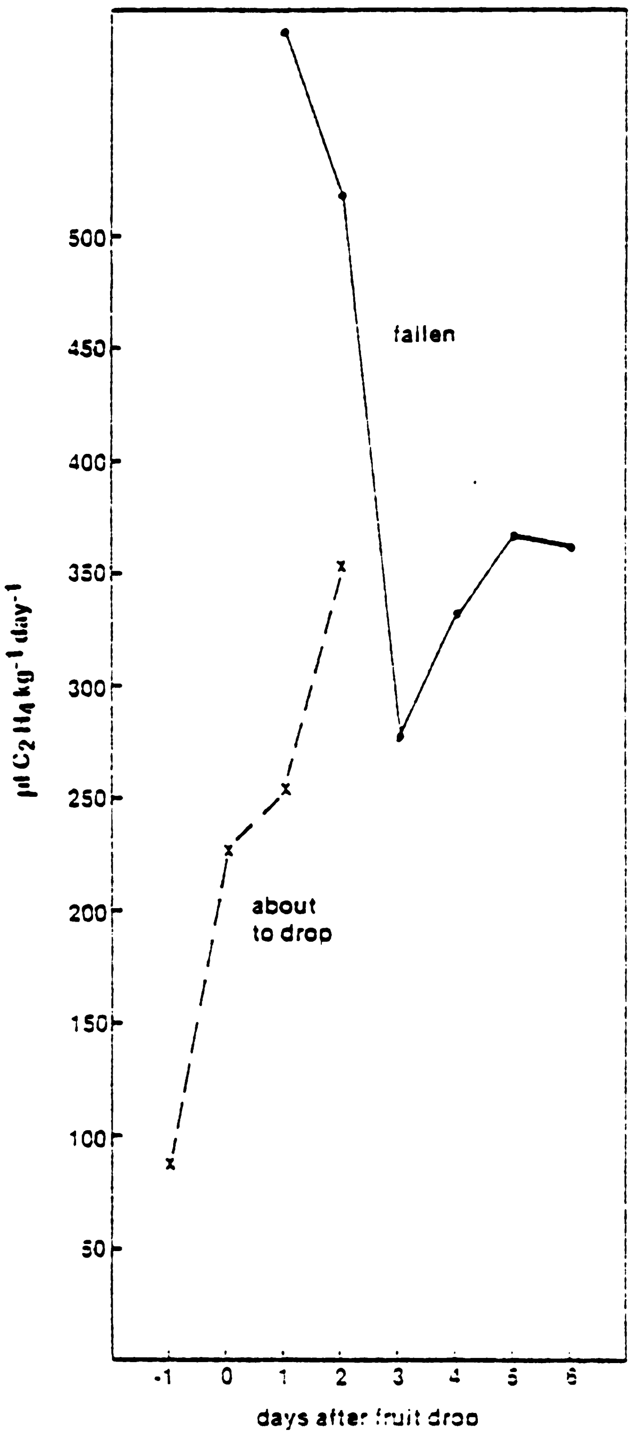


Figure 4: Natural abscission zone and point of calyx attachment in *L. peruvianum* fruit.

Figure 6: Ethylene production in abscised *L. peruvianum* fruits and fruits about to fall. Measurements made daily until softening occurred.


fallen
about
to drop
µl C₂H₄ kg⁻¹ day⁻¹
500
450
400
350
300
250
200
150
100
50
-1
0
1
2
3
4
5
6
days after fruit drop

DISCUSSION

Both *L. chilense* and *L. peruvianum* have fruits that do not ripen, as indicated by softening, until they fall from the vine. Thus the arrival of maturity in these species can be recognized by abscission. Although ripening is accompanied by endogenous ethylene production in both types of fruit, the timing of ethylene production relative to abscission is not the same. In *L. chilense*, ethylene production rises after fruit drop (Figure 3). In *L. peruvianum*, elevated ethylene production precedes abscission (Table II). This implies that the control mechanisms regulating ripening might be different for the two species.

L. chilense: The different patterns of ethylene production in *L. chilense* fruits probably correspond to stages of maturity. Fruits in the first stage, (which includes loss of chlorophyll), produce little ethylene, even when treated with propylene (Figure 2a). The slow loss of chlorophyll, lasting approximately ten days, suggests that active degradation, comparable to the climacteric color-fruited species is not occurring (7). Slow degreening is characteristic of non-climacteric citrus fruits which also do not produce much ethylene (18). This would tempt one to classify *L. chilense* as non-climacteric.

Studies of older fruit, however, show that *L. chilense* is capable of natural ethylene production (Figure 2b).

Treating these fruits with propylene induces a burst of ethylene production. Finally, abscised fruits undergo climacteric ethylene production (Figure 3), and soften in a few days.

Maturation processes in L. chilense fruit are diagramatically summarized by Figure 7. Immature fruits do not naturally produce ethylene, whereas mature fruits do. The final stage in maturation prior to ethylene production and softening is abscission. This behavior is similar to avocado fruit which undergo climacteric ripening after harvest (6). In avocado, ripening is presumably due to the removal of some restraining factor previously supplied by mother plant (6).

L. peruvianum: L. peruvianum fruit are similar to L. chilense: they do not soften until they fall from the vine. Unlike L. chilense, however, the majority of L. peruvianum fruit harvested prior to natural abscission do not soften during storage. Thus detachment itself is not the stimulus for ripening. It is not simply removal of the fruit from the source of inhibitory substance that enables abscised L. peruvianum fruit to ripen.

Wounding is also not responsible for softening that occurs in a minority of hand-detached fruits. If wounding occurred during harvest, it would probably be a result of calyx removal; but some frits have high levels of ethylene production and soften while their calyxes are still attached.

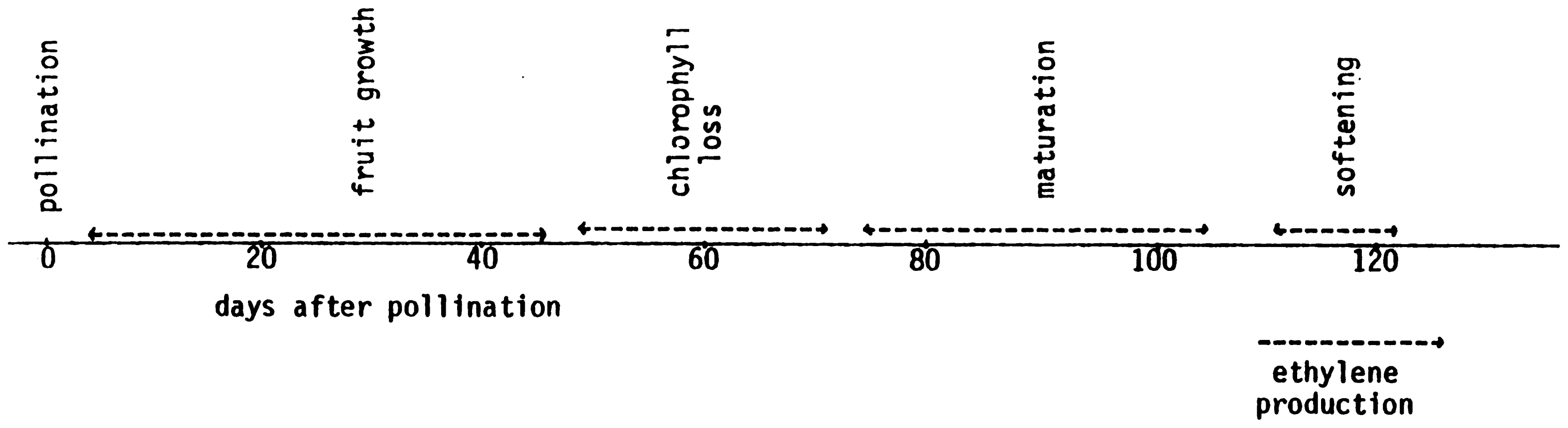


Figure 6: Time-line of developmental processes in *L. chilense* fruit.

In other fruits, calyx removal does not induce an increase in ethylene production, or cause softening.

Fruits that are hand-removed just before they drop soften and produce high levels of ethylene, 300 μl kg^{-1} day^{-1} (Figure 6). Thus physically falling from the plant to the ground and possible subsequent wounding, is also not responsible for ethylene production and softening. Finally, fungal infection is probably not responsible for softening as there was no external sign of infection even when the fruits were held for weeks after becoming soft.

In fruits that have fallen, and those about to drop, an abscission layer must already be formed. It is not detachment or wounding, but probably formation of an abscission layer and concomitant development of ethylene production machinery which stimulates ripening. One would postulate that the minority of hand-detached fruits which soften have already begun abscission zone formation at the time of harvest.

This hypothesis is consistent with models for abscission zone development in leaves (1,13). Abeles et al. (2), working with beans explants, describe a three part process of abscission zone formation as follows: 1) a period of cell aging when ethylene does not promote abscission, 2) the induction of wall-degrading enzymes and synthesis of ethylene production machinery (at this time ethylene enhances abscission) and 3) a loss of break strength due to cell separation.

Perhaps high levels of ethylene are required, either directly or indirectly, to induce softening; and the development of sufficient ethylene production machinery is, in turn, dependent upon cell aging. Thus, in the fruits which are about to fall, or have fallen, ethylene production machinery is well developed. Possibly, hand-detached fruits which soften also have adequate ethylene production machinery and are in stage 3 of abscission zone formation. In these fruits, however, there has not yet been sufficient cellwall degradation to cause fruit drop. In contrast, those fruits which do not soften have not yet produced sufficient ethylene synthesis machinery, and are in stage 1 or 2 of abscission zone formation.

The difference in levels of ethylene production between the two groups of non-softening fruits that were either difficult, or not so difficult, to break at the abscission zone may be because one group is in stage 1 of abscission zone formation (prior to ethylene production), and the other group in stage 2 (synthesis of ethylene production machinery). A possible next experiment would be to take fruits of each category and test for response to propylene. One would predict group 2 fruits to respond by enhanced ethylene production and/or fruit softening and group 1 fruits to be unaffected. These stages of development are summarized diagramatically by Figure 8.

A possible indirect effect of ethylene is to initiate synthesis of a product that can break down a hypothesized

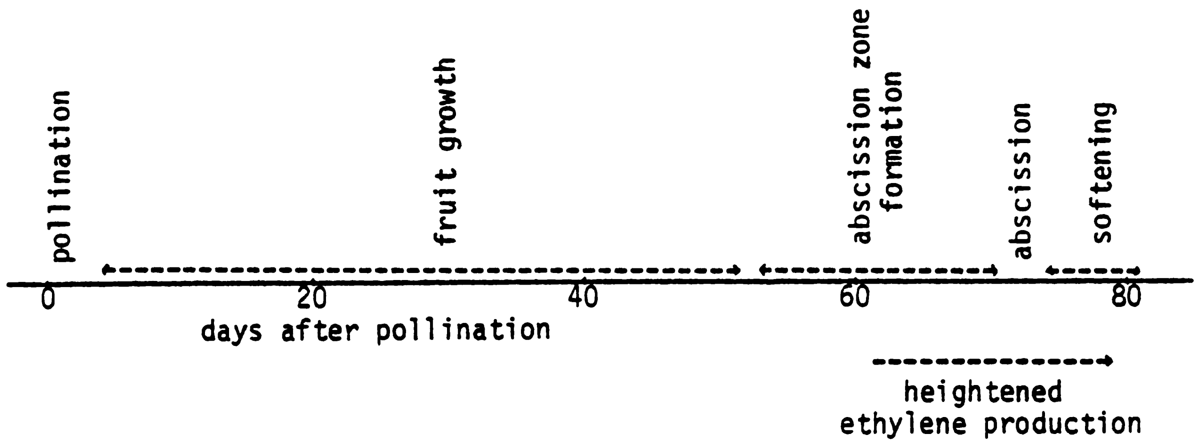


Figure 7: Time-line of developmental processes in L. peruvianum fruit.

inhibitory substance transmitted from the mother plant. This suggestion is consistent with our data. While on the plant there is a continual source of restraining factor, but once the fruit naturally abscise, the factor is degraded. Fruits harvested too young have not yet developed the product necessary for degration of the inhibitory substance, and thus do not soften during storage.

It may be concluded that although fruit of _L_. _chilense_ and _L_. _peruvianum_ both abscise from the plant before ripening, the control mechanisms underlying their behavior are different. Both, however, have mature and immature developmental stages when ripening does or does not occur, respectively. If there is some inhibitory substance involved in the transition of an immature tomato fruit to a mature fruit, further studies with these species may help to characterize the factor and processes leading to its degradation.

LITERATURE CITED

1. Abeles, F. B. 1973. Ethylene in plant biology. Academic Press, New York.

2. Abeles, F. B., L. E. Cracker and G. R. Leather. 1971. Abscission: the phytogerontological effects of ethylene. Plant Physiol. 47:7-9.

3. Abdel-Rahman M., T. H. Thomas, G. J. Doss and L. Howell. 1975. Changes in endogenous plant hormones in cherry tomato fruits during development and maturation. Physiol. Plant. 34:39-43.

4. Dilley, D. R. 1969. Hormonal control of fruit ripening. HortSci. 4:111-114.

5. Frenkel, C. and S. A. Garrison. 1976. Initiation of lycopene synthesis in the tomato mutant rin as influenced by oxygen and ethylene interactions. HortSci. 11:20-21.

6. Gazit, S. and A. Blumenfeld. 1970. Response of mature avacado fruits to ethylene treatments before and after harvest. J. Am. Soc. HortSci. 95:229-231.

7. Grumet, R., J. F. Fobes and R. C. Herner. Ripening behavior in wild, color-fruited tomato species. (in preparation).

8. Grumet, R., J. F. Fobes and R. C. Herner. Ripening behavior in wild, green-fruited tomato species: fruits that ripen on the vine. (in preparation).

9. Hobson, G. E. and J. N. Davies. 1971. The tomato. In A. C. Hulme (ed.) Biochemistry of fruits and their products, Vol. 2. Academic Press, New York.

10. McGlasson, W. B. 1970. The ethylene factor. In A. C. Hulme (ed.) Biochemistry of fruits and their products, Vol. 1. Academic Press. New York.

11. McMurchie, E. J., W. B. McGlasson and I. L. Eaks. 1972. Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 4:451-458.

12. Pratt, H. K. and J. D. Goeschl. 1969. Physiological role of ethylene in plants. Ann. Rev. Plant Physiol. 20:541-584.

13. Rubenstien, B. and A. C. Leopold. 1963. Analysis of the auxin control of bean leaf abscission. Plant Physiol. 38:262-267.

14. Sacher, J. A. 1973. Senescence and post-harvest biology. Ann. Rev. Plant Physiol. 24:197-224.

15. Sawamura, M., E. Knegt and J. Bruinsma. 1978. Levels of endogenous ethylene, CO_2 and soluable pectin and activities of pectin methylesterase and polygalacturonase in ripening tomato fruits. Plant Cell Physiol. 19:1061-1069.

16. Simon, E. W. 1974. Phospholipids and plant membrane permeability. New Phytol. 73:337-420.

17. Tigchelaar, E. C., W. B. McGlasson and R. W. Buescher. 1978. Genetic regulation of tomato fruit ripening. HortSci. 13:508-513.

18. Ting, S. V. and J. A. Attaway. 1971. Citrus fruits. In A. C. Hulme (ed.) Biochemistry of fruits and their products, Vol. 2. Academic Press, New York.

19. Vickery, R. S. and J. Bruinsma. 1973. Compartments and permeability for potassium in developing fruits of tomato. J. Exp. Biol. 24:1261-1270.

CHAPTER III: RIPENING BEHAVIOR OF WILD, GREEN-FRUITED TOMATO SPECIES: FRUITS THAT RIPEN ON THE VINE

ABSTRACT

Fruit of four wild, green-fruited tomato species, L. hirsutum, L. chmielewskii, L. parviflorum and S. pennellii were surveyed for variability in ripening habit. Ripening changes were studied in fruits harvested at different ages; ethylene production and CO_2 evolution were monitored using an ethylene-free flow through system and gas chromatography. Results indicate differences in ripening behavior among the species with respect to ethylene production and respiratory patterns, rate of ripening changes, and whether or not ripening occurs off the vine. Ethylene production in S. pennellii and L. hirsutum was not correlated with external ripening changes, making questionable the role of ethylene as the ripening hormone in these fruits.

INTRODUCTION

Although ripening in the cultivated tomato, Lycopersicon esculentum, has been a topic of much research in recent years, control mechanisms underlying various ripening phenomena are still poorly understood. Most workers agree that ethylene has a central role in triggering the process (1,6,10,11,18), but the inability of ethylene to induce immature tomato fruits to ripen normally indicates that other changes must occur as well. (4,5,7,9).

An unexplored approach to this problem is the use of wild germplasm to study the ripening process. Within the tomato species, a tremendous amount of variability exists for myriad morphological (12) and biochemical characters (12,14-17). Observations in wild habitats have suggested possible inter-generic variation for ripening behavior. The green-fruited tomato species fall into two categories, those that abscise prior to ripening and those that ripen on the vine. The latter category includes *L. hirsutum*, *Solanum pennellii*, *L. chmielewskii* and *L. parviflorum*. All the wild species can be hybridized with the cultivated tomato *L. esculentum*.

L. hirsutum is naturally distributed from south-central Perú to northern Equador, having an altitudinal range from 500-300 m. This robust plant is particularly known for its hairy plant parts and resistance to a variety of insect pests. *S. pennellii* is indigenous to the exceedingly dry, lower slopes of the central Peruvian Andes. It is most outstanding for its ability to withstand drought conditions (17). The two sibling species, *L. chmielewskii* and *L. parviflorum* (17), occupy a restricted area in the interandean region of central Perú; their native distribution is bounded by the Ecuadorian frontier in the north, and the Departamento Cuzco in the south. *L. parviflorum* is distinguished from *L. chmielewskii* by its greatly reduced plant parts and flower size.

MATERIALS AND METHODS

Plant materials: Four wild green-fruited species were characterized for ripening behavior: *L. chmielewskii*, *L. parviflorum*, *L. hirsutum* and *S. pennellii*. They are pictured in Figure 1 and the source of germplasm is listed on Table I. Only one accession of each species was tested. *L. chmielewskii*, *L. parviflorum* and *S. pennellii* and F_1 flowers were selfed by hand so exact fruit age could be determined *L. hirsutum* is self-incompatible, so outcrosses were made for this species. Growing conditions were the same as for Grumet, *et al*. (2).

To determine age at maturity for each species, fruits were harvested every two to three days from the time they reached full size until they indicated some sort of ripening change. The resultant set of 10-12 fruits were monitored for respiration and ethylene production as in Grumet, *et al*. (2). Propylene studies also followed the procedure of Grumet, *et al*, (2).

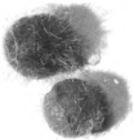
RESULTS

The four green-fruited species in this study *L. chmielewskii*, *L. parviflorum*, *L. hirsutum* and *S. pennellii* "ripen" while attached to the vine. This may be contrasted with *L. chilense* and *L. peruvianum* which do not ripen prior abscission (3). For *L. chmielewskii*, *L. parviflorum*, and *S. pennellii*, ripening is indicated by fruit softening.

Table I: Tomato Germplasm Used for Assay of Ripening Characteristics.

LA no.	Species	Location
1028	*Lycopersicon chmielewskii*	Casinchihua, Apruimae, Perú
247	*L. parviflorum*	Chayanillo Ochoa, Hanúnuco, Perú
1775 C	*L. hirsutum*	Rio Casma, Ancash, Perú
716	*Solanum pennellii*	Atico, Arequipa, Perú

Figure 1: Green-fruited tomatoes that ripen on the vine. Left to right: *Solanum pennellii*, *Lycopersicon hirsutum*, *L. chmielewskii*, *L. parviflorum*.



L. *hirsutum* has no external sign of ripening, but ethylene and respiratory data show this species to have highly regulated climacteric behavior.

S. *pennellii*: The external index of ripening in S. *pennellii* fruit is softening occurring both on and off the vine 70-80 days after pollination. Fruit on the vine become extremely soft and eventually abscise from the plant.

Time to soften in hand-picked fruits is directly correlated with fruit age, once full size has been attained (Figure 2). Once begun, softening occurs quickly. In a period of 2-3 days fruit change from firm to extremely soft, and split open with the slightest pressure. If fruits are kept after they soften, they are susceptible to molds. S. *pennellii* fruit are also very sensitive to water; both immature and mature fruit will split open if washed.

Ethylene production in S. *pennellii* fruit is very erratic. Many observations have shown individual fruits to have several production peaks (Figure 3a); however, there is no peak in production directly corresponding to the onset of fruit softening. Respiratory activity is also erratic and does not correspond to peaks in ethylene production, or to the onset of fruit ripening (Figure 3b). Propylene treatment does not affect ripening in these fruits. Fruit softening, ethylene production, and CO_2 evolution are not altered in the treated group with respect to controls (Figure 3).

Figure 2: Days to ripen vs. age at harvest for _S_. _pennellii_ fruit.

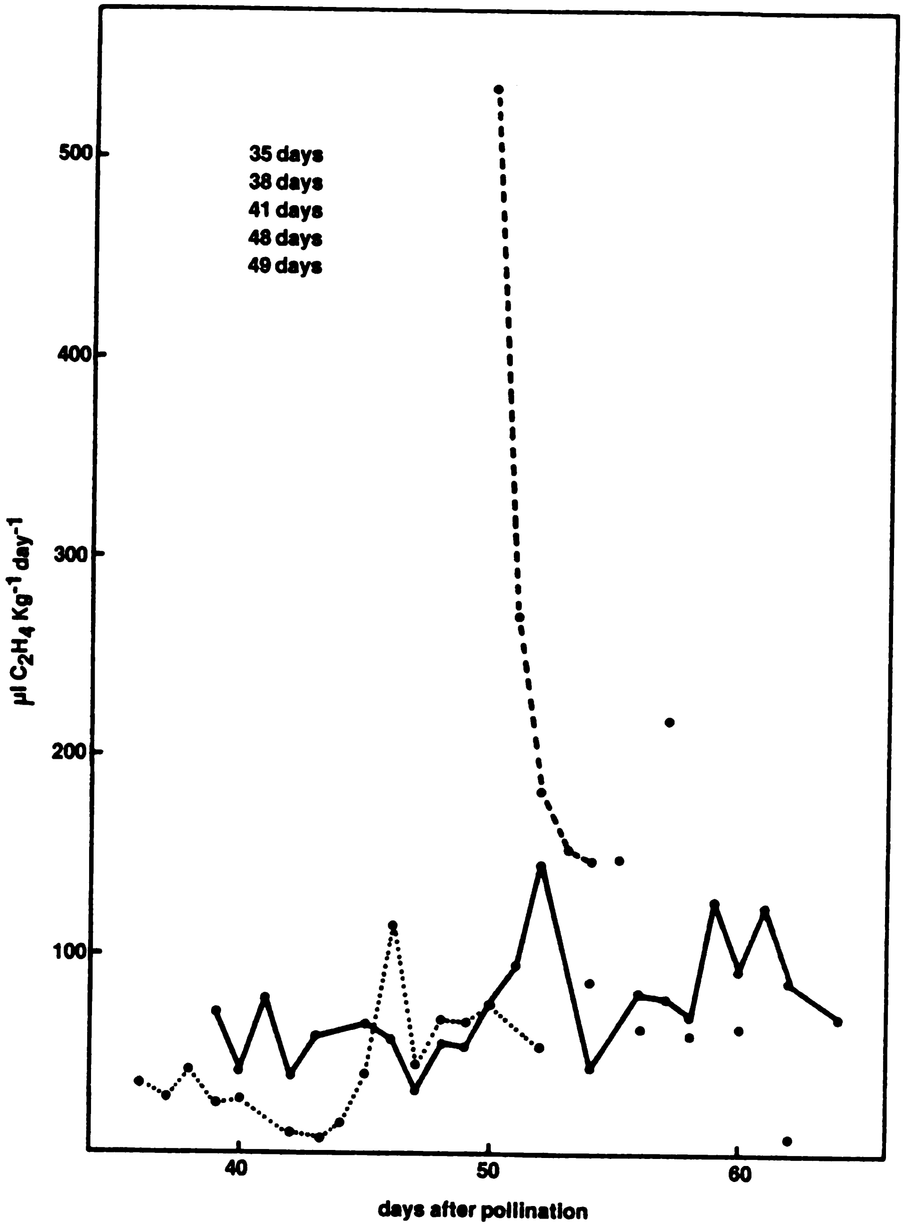
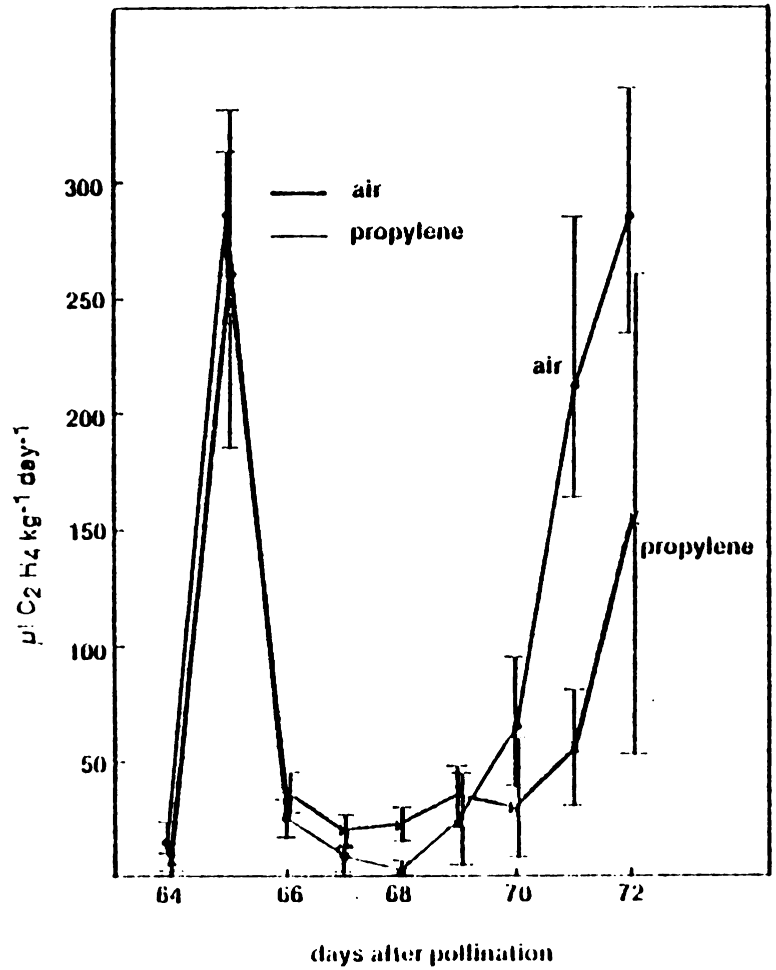
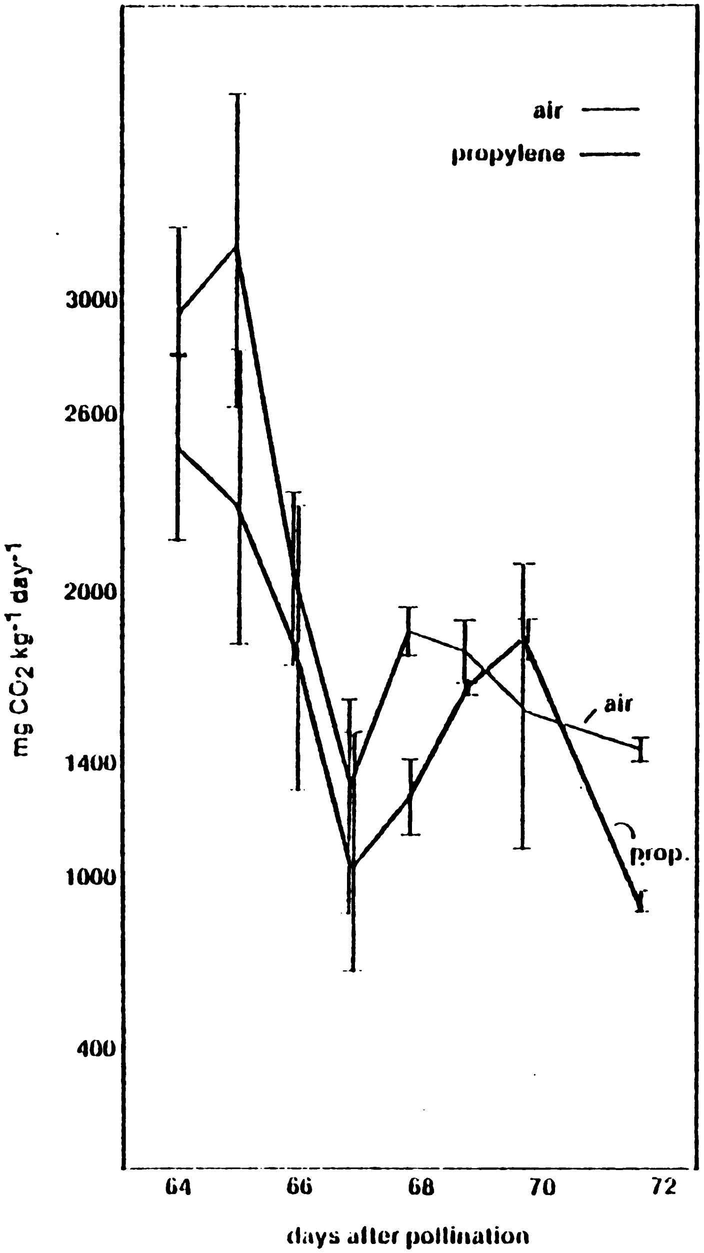

35 days
38 days
41 days
48 days
49 days
500
400
300
200
100
µl C2H4 Kg-1 day-1
40
50
60
days after pollination

Figure 3: Ethylene production (a), respiratory behavior (b), and response to propylene in _S_. _pennellii_ fruit.[1] Softening occurred at 70 days in treated and untreated fruits.

1. data are mean of 3 replicates ± S.E.


air
propylene
air
propylene
µl C_2H_4 kg^{-1} day^{-1}
300
250
200
150
100
50
64
66
68
70
72
days after pollination


air
propylene
air
prop.
mg CO_2 kg^{-1} day^{-1}
3000
2600
2000
1400
1000
400
64
66
68
70
72
days after pollination

L. chmielewskii and L. parviflorum: Ripening, as indicated by fruit softening in the two sibling species, L. chmielewskii and L. parviflorum occurs in mature fruit both on and off the vine. If abscission takes place, it occurs much later than fruit softening. It is possible, however, to harvest these fruits before they are capable of ripening. If harvested too young, they remain hard for more than 30 days in storage; if sufficiently mature when harvested, they soften in 6-12 days (Table II).

L. chmielewskii and L. parviflorum do not flower readily under Michigan conditions. As a result, ethylene and respiratory studies are not sufficiently replicated to be conclusive. From the information obtained to date, however, climacteric behavior is implicated.

The magnitude of ethylene production immediately following harvest for L. chmielewskii fruit is dependent on fruit age (Figure 3). The youngest fruits produce less than 20 µl/kg/day whereas the oldest fruit produce more than 150 µl kg^{-1} day^{-1}.

A set of fruits harvested during the transitional period for ability to soften in storage, 37-48 days after pollination, was studied. They softened 10-12 days after harvest, thereby corresponding with ripening on the vine. Ethylene production declined following harvest and then rose again to what might be considered a climacteric peak.

Ethylene production in L. parviflorum differs in the younger non-softening fruits from the older softening

Table II. Age at Maturity[1] and Days to Soften for Detached *L. chmielewskii* and *L. parviflorum* fruits.

Species	Age at Maturity (days after pollination)	Days to Soften
L. chmielewskii	35-40	6-7
L. parviflorum	38-44	10-12

---

[1] Fruit harvested prior to this age do not soften in storage.

Figure 4: Initial levels of ethylene production vs. fruit age at harvest in _L_. _chmielewskii_ fruit.

fruits. Non-softening fruits harvested prior to 40 days old, have erratic production ranging from 50 to 200 µl kg^{-1} day^{-1}. Fruits harvested at 41 days are mixed; although some fruits softened, ethylene production is relatively low and erratic. Older, softening fruits have climacteric production with peak rates of 400-500 µl kg^{-1} day^{-1}.

L. hirsutum: L. hirsutum fruits have no readily distinguishable, external ripening changes. They remain hard, green and attached to the plant, long after they have undergone climacteric bursts in ethylene production and respiration.

These fruits have an interesting timing mechanism regulating their ethylene production and respiratory behavior. If the fruit are harvested too early, i.e. 40-50 days after pollination, they have steady low levels of ethylene production and low CO_2 evolution, (Figures 5,6). Eventually the fruits wither. If harvested somewhat later, i.e. at 55 days, there is again low ethylene production. This is followed by a small rise that might be termed climacteric. Respiration in these fruits declines throughout storage (Figure 6).

At 58 and 60 days the ethylene peak is increasingly pronounced and respiration also rises. Finally at 65 days the ethylene peak is extraordinary, 2000-4000 µl kg^{-1} day^{-1}. This is ten times peak ethylene production in the cultivated L. esculentum (Table III). Peak respiration in L. hirsutum is three times that of L. esculentum. Fruit harvested at 70 days begin with high ethylene production and decline

Figure 5: Ethylene production in _L_. _hirsutum_ fruit harvested at different ages.

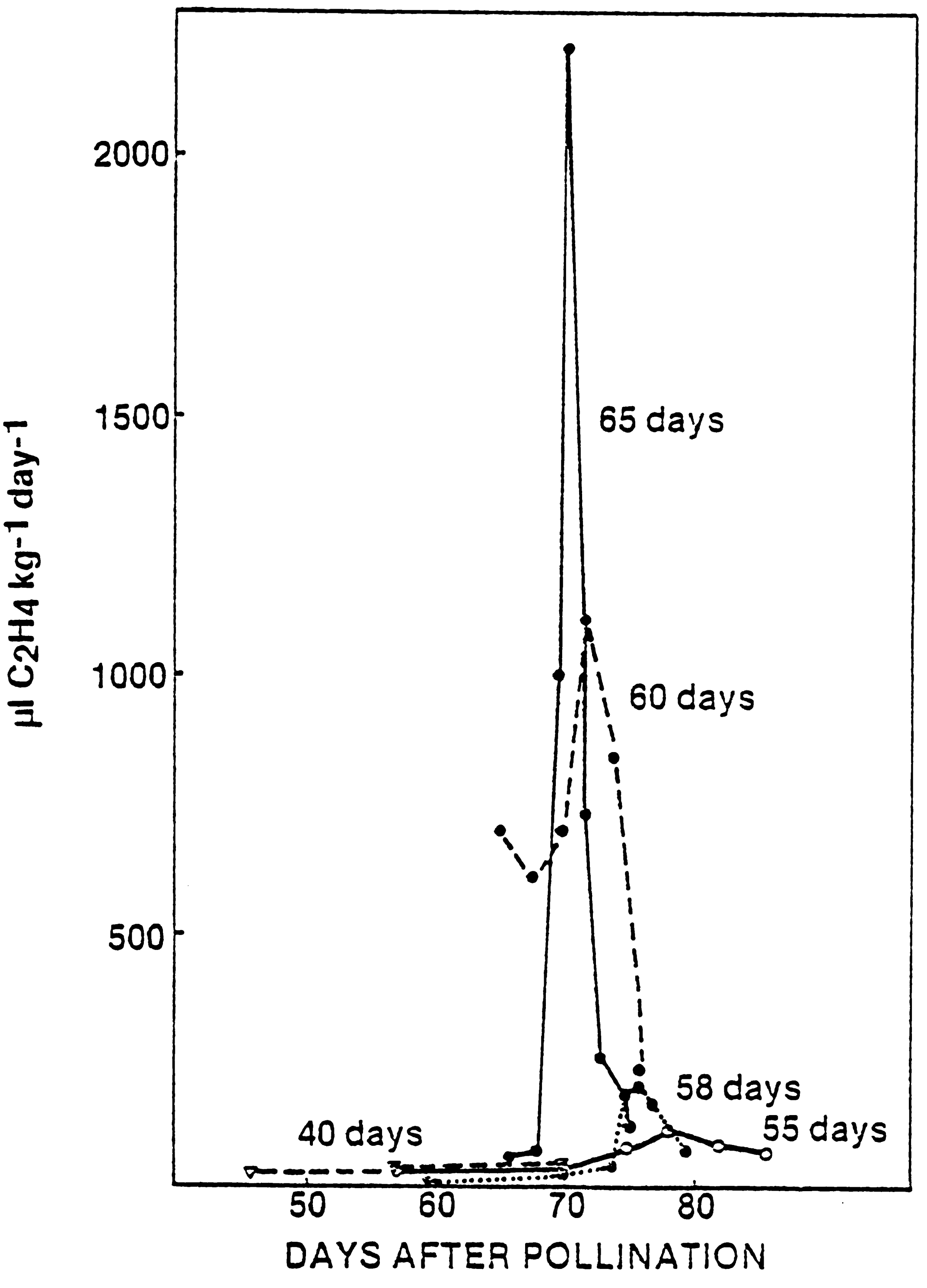

µl C2H4 kg-1 day-1
2000
1500
1000
500
65 days
60 days
58 days
55 days
40 days
50
60
70
80
DAYS AFTER POLLINATION

Figure 6: Respiration in *L. hirsutum* fruit harvested at different ages.

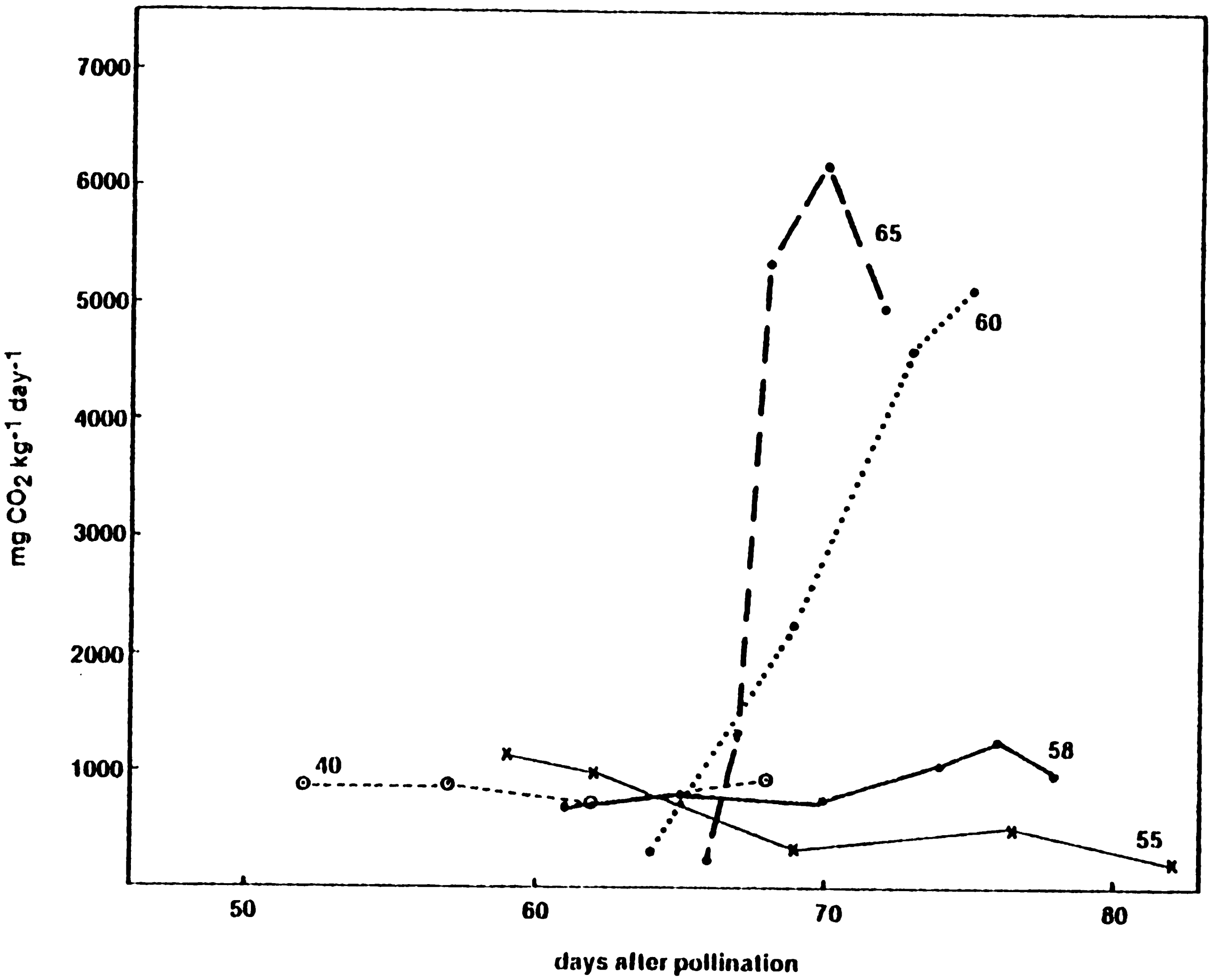

mg CO_2 kg^{-1} day^{-1}
7000
6000
5000
4000
3000
2000
1000
50
60
70
80
days after pollination
65
60
58
55
40

Table III: Comparison of Peak Rates of Ethylene Production and CO_2 Evolution in *L. hirsutum* Fruit with those of Climacteric, Color Fruited Tomatoes.

Species	µl kg^{-1} day^{-1} Ethylene Production	mg kg^{-1} day^{-1} CO_2 Evolution
L. pimpinellifolium	75-150	800-1000
L. esculentum var. *cerasiforme*	200-250	1000-2000
L. esculentum cv. VF36	200-250	1000-2000
L. cheesmanii	300-400	3000-4000
L. hirsutum	2000-4000	6000-7000

[1] Color-fruited data from Grumet *et al*. (2)

rapidly. Fruit harvested even later have low levels of ethylene production and respiration.

Figures 5 and 6 show that harvesting too early not only diminishes climacteric response, but delays it as well. Fruits harvested later exhibit peak production sooner than young fruits. These patterns for ethylene production and respiratory behavior have been replicated three times. For fruits grown in the winter the timing is shifted later by a few days, but the pattern reamins the same.

DISCUSSION

In addition to exhibiting diversity for visible ripening changes, *L. parviflorum*, *L. chmielewskii*, *L. hirsutum* and *S. pennellii* also exhibit variability for other physiological traits associated with the ripening process. Such traits include ethylene evolution, internal timing mechanisms, and whether or not fruit remain incapable of ripening for some time after attaining full size.

S. pennellii: Based on ethylene, respiratory and propylene data, it is difficult to conclude that ethylene plays a key regulatory role in ripening of *S. pennellii* fruit. Ethylene is produced by detached fruit, but there is no single climacteric peak, production levels do not correspond to softening and subsequent abscission of mature *S. pennellii* fruit, however, as well as the negative linear relationship between fruit age at harvest and time to ripen, indicates that some control mechanisms must be involved. Ethylene may play a role in regulation of softening and abscission for fruits attached to the vine, but this cannot be determined from our studies.

L. chmielewskii and L. parviflorum: It is possible to harvest L. chmielewskii and L. parviflorum fruits after they have attained full size, but before they are capable of ripening. The failure of younger fruits to ripen after harvest suggests that some maturation process must occur while attached to the plant that cannot take place off the plant. This is similar to behavior of the cultivated tomato (5). There, too, it is possible to harvest a fruit before it is fully capable of ripening, although it may have already attained full size (5).

Preliminary ethylene data for L. chmielewskii fruit show the magnitude of production following detachment to be correlated with fruit age (Figure 4), thus one might expect ethylene production in harvested fruit to continue to increase as the fruit ages. In fruit harvested 37-41 days after pollination, however, ethylene production declines following harvest and the subsequent climacteric peak remains within the range of initial ethylene production levels.

For L. parviflorum, it is interesting to note, that although younger fruit are capable of producing quite large amounts of ethylene, they do not soften. Either those amounts are not sufficient for this species, or some factor other than ethylene is induced. For both species, additional replication is needed before conclusions can be drawn.

L. hirsutum: *L. hirsutum* fruit are very unusual. Their ethylene production and respiratory behavior are typically climacteric, but show extremely precise timing. There is a very specific age when detached fruits produce large quantities of ethylene and have corresponding heightened respiration (Figures 5,6). If fruits are harvested too young, they show no, or minimal response. If harvested too old, they also do not exhibit climacteric behavior. The time difference between too young and too old for peak production is only a few days. An interesting experiment might be to harvest fruits daily, or to monitor ethylene and CO_2 evolution of fruits attached to the vine.

These patterns of ethylene and CO_2 evolution are also outstanding because the peak rates of evolution of both gasses are much greater than for any other tomato species (Table III). Additionally, the peak rates do not coincide with any external ripening changes. Apparently, in this case ethylene does not serve as a trigger for ripening. Whether or not the ethylene production is correlated with seed maturation is unknown.

To conclude, although *L. chmielewskii*, *L. parviflorum*, *L. hirsutum* and *S. pennellii* all "ripen" while attached to the vine, there are differences in the regulatory mechanisms involved. Softening in *S. pennellii* fruit occurs very quickly and is shortly followed by abscission. This closely synchronized behavior suggests some sort of regulatory mechanism. Whether ethylene plays a key role is questionable because peaks in ethylene production are not directly correlated to fruit softening.

In contrast to *S. pennellii*, *L. chmielewskii*, and *L. parviflorum* fruit take a relatively long time to soften. If abscission occurs at all, it takes place much later than fruit softening. Thus ripening processes are not as tightly integrated in these fruits. However, the inability of young detached fruits to soften suggest that there are control mechanisms responsible for fruit maturation on the vine.

Finally, *L. hirsutum* has an extremely specific timing mechanism regulating its climacteric behavior despite the absence of resultant external ripening change, As in *S. pennellii* the physiological significance of ethylene production in these fruits is a mystery.

LITERATURE CITED

1. Abeles, F. B. 1973. Ethylene in plant biology. Academic Press. New York.

2. Grumet, R., J. F. Fobes and R. C. Herner. Ripening behavior in wild, color-fruited tomato species. (in preparation).

3. Grumet, R., J. F. Fobes and R. C. Herner. Ripening behavior in wild, green fruited tomato species: fruits that abscise prior to ripening. (in preparation).

4. Herner, R. C. and K. C. Sink. 1973. Ethylene production and respiratory behavior of the *rin* tomato mutant. Plant Physiol. 52:38-42.

5. Kader, A. A., M. A. Stevens, M. Albright-Holton, L. L. Morris and M. Algazi. 1977. Effect of fruit ripeness when picked on flavor and composition in fresh market tomatoes. J. Amer. Soc. Hort. Sci. 102:724-731.

6. McGlasson, W. B. 1970. The ethylene factor. *In* A. C. Hulme (ed.) Biochemistry of fruits and their products, Vol. 1. Academic Press, New York.

7. McGlasson, W. B. and I. Adato. 1977. Relationship between the capacity to ripen and ontogeny in tomato fruits. Aust. J. Plant Physiol. 4:451-458.

8. McMurchie, E. J., W. B. McGlasson and I. L. Eaks. 1972. Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237:235-236.

9. Ng, T. J. and E. G. Tigchelaar. 1977. Action of the non-ripening (*nor*) mutant on fruit ripening of tomato. J. Amer. Soc. Hort. Sci. 102:505-509.

10. Pratt, H. K. and J. D. Geoschl. 1969. Physiological role of ethylene in plants. Ann. Rev. Plant Physiol. 20:541-584.

11. Rhodes, M. J. C. 1970. The climacteric and ripening of fruits. *In* A. C. Hulme (ed.) Biochemistry of fruits and their products, Vol. 1. Academic Press, New York.

12. Rick, C. M. 1973. Potential genetic resources in tomato species: clued from observations in native habitats. In A. M. Serb (ed.) Genes, enzymes and populations. Plenum Press, New York.

13. Rick, C. M. 1975. The tomato. In R. C. King (ed.) Handbook of genetics, Vol. 2. Plenum Press, New York.

14. Rick, C. M. and J. F. Fobes. 1975. Allozymes of Galapagos tomatoes: polymorphism, geographic distribution and affinities. Evolution 29: 443-457.

15. Rick, C. M. and J. F. Fobes. Allozyme variation in the cultivated tomato and closely related species. Bull. Torrey Bot. Club 102:376-384.

16. Rick, C. M., J. F. Fobes and M. Holle. 1977. Genetic variation in Lycopersicon pimpinellifolium: evidence of evolutionary change in mating systems. Plant System. and Evol. 127:139-170.

17. Rick, C. M. and E. Kesicki, J. F. Fobes, and M. Holle. 1976. Genetic and biosystematic studies on two new sibling species of Lycopersicon from interandean Peru. Theoret. Appl. Genet. 47:55-68.

18. Sacher, J. A. 1973. Senescence and post-harvest biology. Ann. Rev. Plant Physiol. 24:197-224.